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ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOR PATIENTS WITH TREATMENT-REFRACTORY CROHN'S DISEASE: A PHASE 2 STUDY

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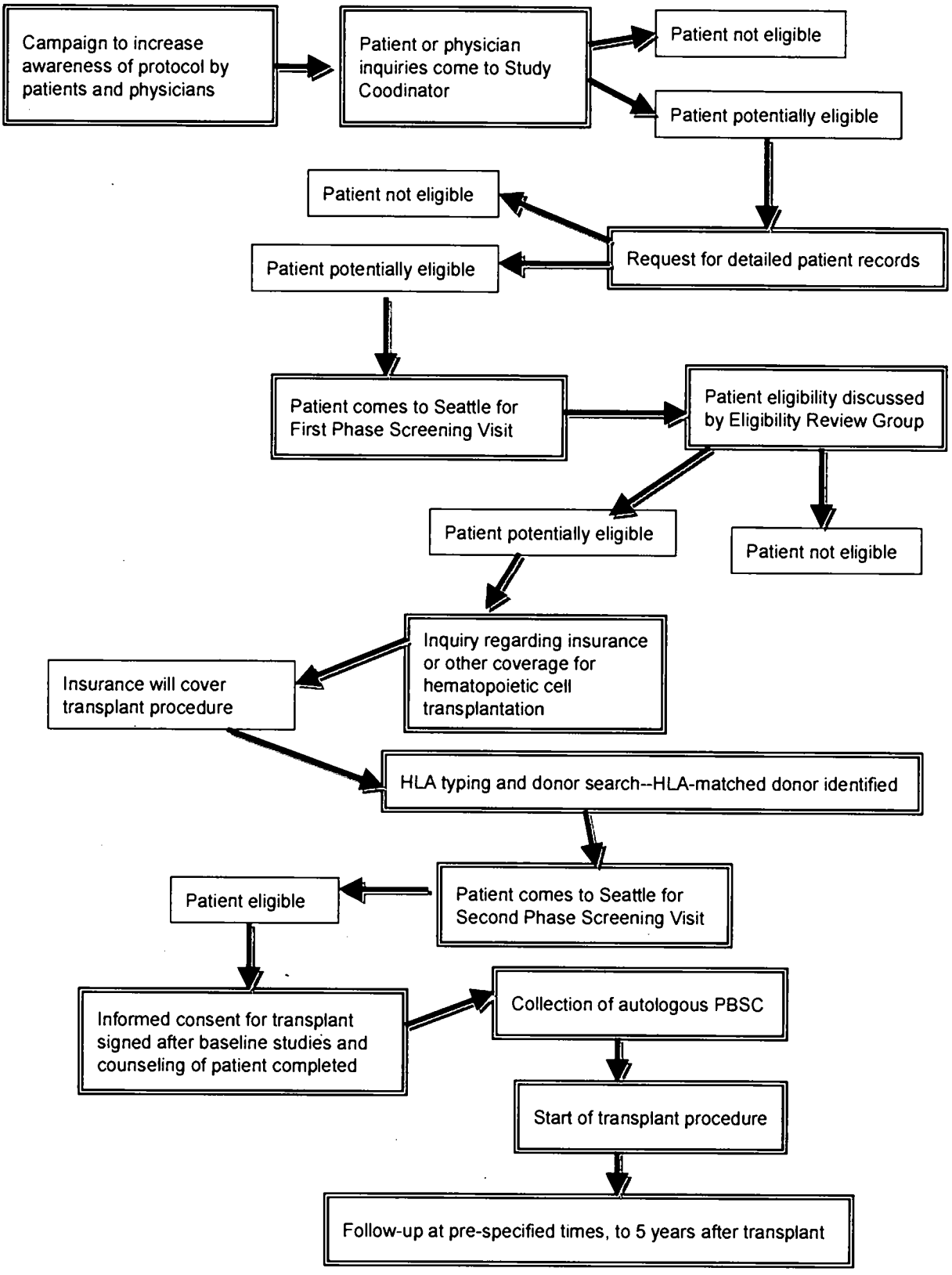

1.0 INTRODUCTION

This document describes a clinical research study to be conducted in compliance with the Fred Hutchinson Cancer Research Center's IRB approved protocol, associated Federal regulations, all applicable IRB requirements, and the U.S. Food and Drug Administration Investigational New Drug application #15081. The rationale for this study is as follows: Genetic and epidemiologic evidence suggests that Crohn's Disease (CD) is an inherited disorder of immune dysregulation [1-4]. By providing patients with an immune system from a donor without CD or genetic predisposition to CD, we predict that CD would disappear and not recur in the allografted patient. The hypothesis to be tested is that allogeneic hematopoietic cell transplantation (HCT) can achieve complete, sustained remissions in patients with treatment-refractory CD.

2.0 BACKGROUND

2.1 Crohn's Disease

CD is an inflammatory disease of the gastrointestinal tract that affects mostly the distal small intestine and colon, but which can affect mucosa from the mouth to the anus as well as extraintestinal tissues [1]. CD inflammation can be discontinuous and involve all intestinal layers (that is, there can be transmural involvement of segments of the intestine). The most common symptoms are diarrhea, weight loss, abdominal pain, and fever, sometimes complicated by fistulas and intestinal strictures that may require surgery. Continued active disease is frequently accompanied by malaise, decreased energy, depression, body image concerns, and poor psychosocial function and quality of life. Children and adolescent patients with CD often have the additional morbidity of abnormal growth, sexual maturation, and psychosocial development. The natural history of CD varies among patients, with some patients having a relatively indolent course but others have aggressive fistulizing disease or recurrent intestinal structuring and inflammation. There are no curative treatments, but therapy is usually effective in lessening the severity of symptoms. However, despite recent advances in biologic therapies, there remains a cohort of patients with CD whose disease is either refractory to therapy, or recurs after each surgery or therapy. These refractory patients are the intended subjects for the phase 2 study of allogeneic HCT proposed here.

2.1.1. Pathogenesis: Genetics and Environment. There is strong evidence for genetic susceptibility to inflammatory bowel disease (IBD) in general and CD in particular [2] [Elding H, et al, Refinement in localization and identification of gene regions associated with Crohn Disease. Am J Human Genetics 2013; 92: 1-7-113]. Recent refinement in identification of gene regions associated with CD has provided evidence for 200 such regions, confirming that CD is a complex, polygenic disorder. Family members of patients with IBD are at increased risk of developing IBD, with a 15-fold relative risk among first degree relatives, and 20% of patients with CD have at least one affected relative [5]. Ashkenazi Jews have a 2-4-fold increased risk of developing IBD than non-Jews in the same geographic location and are at greater risk of having multiple family members with IBD. The concordance rate among monozygotic twins has been reported to be as high as 67% for CD. More than 70 susceptibility loci have been convincingly identified with CD in Genome-Wide Association (GWA) studies [3]; four pathways related to disease pathogenesis have emerged:

1. One pathway involves the defective sensing of intracellular bacteria and reduced production of β-defensins, antibacterial proteins secreted by intestinal Paneth cells, through allelic variants in the NOD2/CARD15 gene. NOD2 is expressed by lymphocytes, macrophages, antigen-presenting cells, Paneth cells, fibroblasts, and epithelial cells. Activation of NOD2 by microbial ligands activates NF-κB and protein kinase signaling, functioning as a positive regulator of immune defense. The presence of variant genes on both chromosomes yields an odds ratio of 17.1 (10-7 – 27.2) for CD, with heterozygotes having an OR of 2.5 (2.0 – 2.9). Variant NOD2/CARD15 genes are found in 20 – 30% of patients with CD, but the penetrance of variant genes is <1% suggesting that environmental factors are important in disease expression [2,6].

2. A second pathway with a genetic link to CD involves the processes of autophagy and xenophagy, processes that clear intracellular proteins and bacteria and integrate innate and adaptive immune responses [7,8] Autophagy 16-like 1 (ATG16L1) is strongly associated with CD; it is expressed in mucosal Paneth cells and mediates exocytosis of cryptidins (antibacterial peptides). Genes that encode immunity-related guanosine triphosphatase M (IRGM) and leucine-rich repeat kinase 2 (KRRK2), regulators of autophagy, are also associated with CD in GWAS studies.

3. A third pathway associated with CD involves the IL-23 gene and gene products. A rare Single Nucleotide Polymorphism (SNP) in the IL23R gene is strongly protective for CD. Other, more common SNPs confer increased risk of CD. Variants of the IL12B, the JAK2, and STAT3 genes, with roles in IL23R signaling and Th17 differentiation, are associated with susceptibility to CD.

4. A fourth pathway involves lymphocyte activation, survival, and growth. Meta analyses have demonstrated stronger associations between HLA class II and class I genes and ulcerative colitis than between these genes and CD. Nonetheless, *DRB1*0410*, *DQB*0401*, and *DRB1*0103* confer risk for CD (odds ratios 3.9, 2.8, and 2.07, respectively); among class I genes, *Cw8* and *B21* confer risk for CD (ORs 3.4 and 2.3, respectively) [9].

Environmental factors appear to interact with genetic susceptibility in leading to CD. This conclusion is derived from data showing that identified susceptibility loci in GSAS studies account for <25% of predicted heritability [3]; the low penetrance of the NOD2/CARD15 gene; and epidemiologic studies that have identified risk factors for CD vs. controls without CD [10]. These epidemiologic studies implicate as risk factors for CD white-collar work status (as compared to outdoor physical labor), a diet with few fresh fruits and vegetables, use of NSAIDS, and smoking. Breast feeding during infancy may be protective. More convincing data for environmental influences come from studies showing geographic variation in CD prevalence (higher in northern latitudes), rising incidence rates in some countries across time, and studies of migrant populations [10].

2.1.2. Pathogenesis: Microbes, epithelial function, and innate immunity. The proximate cause of intestinal inflammation in CD is thought to be microbial in origin, with higher numbers of mucosa-associated bacteria and fewer varieties of commensal bacteria, but it is not entirely certain which comes first, chicken (abnormal mucosa/immunity) or egg (abnormal microbes) [11]. There is evidence for altered mucosal epithelial cell function as an early event in CD, related to abnormalities in autophagy and intracellular bacterial sensing (interacting pathways in dendritic cells, intestinal epithelial cells, and Paneth cells), and unfolded protein responses [7]. Normally, the innate immune system senses microbes, and epithelial cells, dendritic cells, and macrophages respond initially, with the overall effect being one of immune tolerance to microbes, with disease being a result of loss of tolerance [12].

2.1.3. Pathogenesis: Adaptive Immune responses. Adaptive immunity involving the IL12/IL23 pathway appears to be deranged in patients with CD, with a shift to Th1 and Th17 responses, in turn upregulating effector T cell responses. Defects in regulatory T cells may also affect the inflammatory cascade. Alterations in the function of dendritic cells in maintaining mucosal tolerance leads to expression of TNFα within the mucosa, resulting in a cascade of pro-inflammatory cytokines. Mucosal macrophages and dendritic cells also activate T cells through antigen presentation and a co-stimulatory signal. Leukocyte trafficking amplifies the mucosal immune response.

2.1.4. Clinical presentation, complications, and natural history of CD. Although CD can affect any part of the gastrointestinal tract, the most common sites are the terminal ileum and right colon; a third of patients have disease confined to the small intestine; and some have only colonic involvement. Esophageal, gastric, and duodenal CD are rare, but if present, there is almost always ileal or colonic disease. The clinical presentation is highly variable, some patients presenting with only malaise, weight loss, and fever and others with symptoms that point clearly to the gut as the source--abdominal pain, diarrhea, bleeding, and symptoms of obstruction. In children, growth retardation and weight loss can be presenting symptoms; when there is anorexia, nausea, and vomiting in a younger patient, gastroduodenal CD should be suspected. Perianal disease usually occurs at or after the onset of gut symptoms, but perianal disease may be the initial CD presentation. Fistulas and strictures are highly characteristic of CD and cause considerable morbidity independent of ongoing mucosal inflammation. Perianal fistulas develop in 15-35% of patients and can be extensive. Fistulas from one segment of the gut to another and from the gut to adjacent organs (vagina, bladder, abdominal wall) are also common. It has been estimated that a quarter of patients with CD will have an intra-abdominal abscess sometime during the course of the disease [13]. Stricturing of segments of the gut involved with CD are also common causes of obstruction, presenting with pain and bloating after meals. Surgical resection and stricturoplasty are operations for intractable symptoms or complete obstruction. However, it may be difficult to differentiate between an inflammatory narrowing and one with irreversible fibrosis.

Aside from gut symptoms, there are numerous complications of CD that can be debilitating. Weight loss is usually due to poor oral caloric intake and not to malabsorption of macronutrients, although extensive small

intestinal involvement and enteric fistulas can lead to malabsorption of both macro- and micronutrients. Malaise, lack of energy, and low grade fever are common problems in patients with active inflammation. Anemia can be due to blood loss, folate or B12 deficiency, or inflammatory block of erythropoiesis. Other problems in patients with long-term CD include cholesterol gallstones in patients with ileal resections and oxalate renal stones in those with fat malabsorption (enteric hyperoxaluria); arthritis, arthralgias, osteopenia, and osteoporosis; the skin lesions pyoderma gangrenosa and erythema nodosum; eye involvement with episcleritis, scleritis, and uveitis; and rarely, heart and lung inflammation.

"What is past, is prologue" applies to prognosis in patients with CD. In the first year after initial diagnosis, the cumulative rate of relapse of active disease is ~50%. Active disease in one year predicts a high likelihood of active disease in the next year. However, among those with a diagnosis of CD whose disease has been quiescent for a year, 80% will remain in a quiescent state for the next year; 22% will remain quiescent for the subsequent four years; 25% will have recurrent and persistent symptoms; and 53% will fluctuate between active CD and quiescent disease [14]. Serologic markers (antibodies to microbial and carbohydrate antigens) are predictive of more complicated CD and more rapidly progressive disease. If polymorphisms are present in the NOD2/CARD15 gene, the likelihood of intestinal stricture formation is higher [15-18].

2.1.5. Diagnosis and differential diagnosis. The diagnosis of CD may be difficult at the onset of symptoms, but once a finding of intestinal mucosal inflammation is confirmed by imaging or histology, the differential diagnosis narrows to other forms of inflammatory bowel disease (especially ulcerative colitis), NSAID medications, ischemic bowel disease, connective tissue diseases, diffuse malignant disorders (lymphoma, carcinoma), diverticular disease, and infections of the gut, particularly in patients with immune deficiency, either congenital or acquired. The gold standard for the diagnosis of CD is consistent findings in the clinical presentation, physical examination, radiologic and ultrasound imaging, endoscopic appearance, and histology of mucosal biopsy or surgical specimens. No single test, sign, or symptoms is diagnostic of CD. When the known natural history of CD is played out over many years in an individual patient, one can be more confident that the diagnosis of CD is secure.

In a patient with a consistent history, physical examination, and laboratory studies, the diagnosis is usually confirmed by imaging the gut indirectly (barium contrast x-rays, CT enterography, sono-contrast ultrasound, MR enterography, radionuclide imaging) or directly (endoscopic visualization). The accuracy of indirect imaging in making the diagnosis of CD has improved markedly with the advent of more sensitive methods [19]. When compared to a consensus diagnosis based on clinical and all available imaging methods, CT enterography had a sensitivity of 82%, a specificity of 89%, and accuracy of 85% [20]. MR enterography had a diagnostic accuracy of 91%, compared to a diagnosis based on ileoscopy/colonoscopy as the gold standard [21]. In children with suspected CD, leukocyte scintigraphy with ^{99}Tc had an accuracy of 84% compared to intraoperative findings [22]. PET/CT may have similar diagnostic capabilities.

Histological examination of mucosal biopsy specimens and surgically-resected segments of intestine play an important role in defining the presence of inflammation and in differentiating CD from other causes of inflammation. No one histologic feature, however, is sensitive and specific for CD. Findings of focal inflammation of crypts, intense focal inflammation in the lamina propria, and aphthae and ulcers adjacent to normal mucosa are typical of CD. The presence of granulomas in mucosal biopsy specimens is highly characteristic of CD, but not specific or sensitive as a diagnostic test. CD granulomas are composed of epithelioid histiocytes and inflammatory cells, usually scattered in the lamina propria. Later histologic findings typical of CD include coalescence of aphthae into larger, serpiginious ulcers that can lead to a cobblestone appearance of the mucosa. Transmural inflammation, while typical of CD, can be focal in distribution. Larger ulcers, sinus tracts, and strictures are features of CD of longer duration. Sinus tracts end blindly, while fistulas enter adjacent organs (bowel, bladder, vagina, skin). Fibrosis can be seen as irregular thickening of the bowel wall and may contribute to strictures.

Almost all patients with CD come to endoscopic evaluation, including biopsies of both abnormal and normal-appearing mucosa. Typical endoscopic features of CD include mucosal edema, aphthous ulcers, cobblestone appearance to mucosa, luminal narrowing, lesions within normal-appearing mucosa, discontinuous involvement, abnormal mucosa in the terminal ileum. Many of these features differ from those in typical ulcerative colitis, but there is a category of IBD termed "indeterminate colitis", in which histologic features do not permit separation of CD from UC, and these cases comprise about 10% of patients presenting with IBD. The discriminant features of CD (vs. a diagnosis of UC or indeterminant colitis) include small intestinal mucosal inflammation, mostly right-sided colon inflammation, rectal sparing, fistulas, perineal complications, and granulomas on histology. In cases where the diagnosis is not clear, the passage of time often allows the diagnosis of CD to become more secure

when there is accumulation of additional findings typical of CD. In addition, separation of CD from UC may be easier when histologic features can be evaluated in a resection specimen, where transmural inflammation and fistulas are more evident than on small biopsy samples.

At the onset of symptoms, when there may be diagnostic uncertainty about the precise diagnosis, examination of a panel of immunologic markers (pANCA, ASCA, anti-OmpC, anti-CBir1) can provide additional evidence for or against the diagnosis of CD. When there is a high prevalence of CD in the study population, the Positive Predictive Value (PPV) and Negative Predictive Value (NPV) for a diagnostic algorithm incorporating these markers is 96% and 93% for CD, respectively; these results fall to 74% and 73% in a low-prevalence situation [1].

2.1.6. Assessment of severity of disease. For research purposes, particularly when comparative drug studies attempt to measure and compare treatment responses, several composite scoring systems have been devised, combining symptom assessment with objective parameters of disease activity (findings on examination and laboratory results). Although these scoring systems have their flaws (for example, some degree of interobserver variation and symptoms that reflect obstruction rather than intestinal inflammation), they are widely used. The most common systems, ones that will be used in this protocol, is the Crohn's Disease Activity Index (CDAI) and the Harvey-Bradshaw Index [Vermeire S, et al. Correlation between the Crohn's disease activity and Harvey-Bradshaw indices in assessing Crohn's disease activity. Clin Gastroenterol Hepatol 2010; 8: 357-363], or, for pediatric patients, the Pediatric CD Activity Index (PCDAI), Appendix A); on-line calculators are available for computation (e.g., http://www.ibdjohn.com/cdai/ and http://gastro.cchmc.org/calculators/pcdai/ , respectively).

Measurements of laboratory parameters reflecting intestinal inflammation have been used to assess CD activity, but in general these measures lack sensitivity and specificity. These parameters include Erythrocyte Sedimentation Rate (ESR), C-reactive Protein (CRP) and orosomucoid which in turn reflect serum levels of pro-inflammatory cytokines; fecal calprotectin and lactoferrin; and quantification of radiolabeled leukocytes [23,24]. Histologic evaluation of mucosal biopsy specimens, along with studies of immune activation in these specimens, can be accurate if biopsies are well-targeted and a standardized approach to processing and evaluation is followed.

Because CD is a disorder that causes considerably more morbidity than it does mortality, measures of health-related quality of life instruments have been included in treatment protocols. The instruments in common use for adult patients are the Inflammatory Bowel Disease Questionnaire (IBDQ) and the Short Inflammatory Bowel Disease Questionnaire (SIBDQ, Appendix B), which incorporate elements of social, systemic and emotional symptoms, as well as bowel related symptoms into an activity index. The IBDQ and SIBDQ correlate well with the CDAI in assessing disease activity and are accurate in quantifying the effect of symptoms on perceived quality of life. [25,26]. For pediatric patients, the comparable instruments are the Pediatric CDAI and the Impact-III QOL instrument [27-29]. Several endoscopic scoring systems for the extent of CD lesions have been developed; the system to be used in this protocol is the Simple Endoscopic Score for CD (SES-CD, Appendix C) [30].

2.1.7. Treatment. As there is no current curative therapy, the goals of treatment are to treat active CD to a state of symptomatic quiescence, and to maintain these symptomatic remissions, while avoiding harm from treatment. Maintaining optimal nutritional status and, in children, achieving normal growth and development are important additional goals. Methods of treatment fall into five general categories: standard medical therapy, biologic response modifier therapy, surgical approaches, nutritional approaches, and novel therapies [1,31,32].

> ***Standard medical therapy*** includes aminosalicylates, antibiotics, glucocorticoids, thiopurines, methotrexate, and other immune modulators, as reviewed in [1] and [31]. Briefly, sulfasalazine is useful for treating patients with mild to moderate colonic CD. Antibiotics (metronidazole, ciprofloxacin, clarithromycin, rifaximin) have been used for treating perineal CD, fistulas, active colonic CD, and as post-surgical prophylaxis, but randomized trials have not shown these drugs to offer long term remissions, and biologic response modifiers have largely supplanted antibiotics for these indications. Systemic glucocorticoids are very effective in achieving short- term remissions, but have an adverse toxicity profile, especially when used in higher doses, for prolonged periods of time, and with biologic response modifiers. Oral topical glucocorticoids (budesonide, beclomethasone dipropionate) have similar short-term effects as systemic glucocorticoids for milder disease, but neither systemic nor topical glucocorticoids are suitable for long-term maintenance therapy. The thiopurine antimetabolites azathioprine and 6-mercaptopurine have been extensively used for treatment of active CD in whom glucocorticoids fail, or in those who cannot be tapered off glucocorticoids without a flare, or in those who require glucocorticoid-sparing long-term therapy to prevent flares. Methotrexate is used as an alternative to thiopurine drugs in patients who are intolerant or

unresponsive; monthly pulsed doses of cyclophosphamide are also used for this indication [33,34]. Both thiopurines and methotrexate are used as glucocorticoid-sparing agents in long-term use. Calcineurin inhibitors and mycophenolic acid-related drugs may have a role in treating glucocorticoid-resistant CD.

Biologic response modifier therapies fall into three general classes—anti-TNFα, anti-α_4-integrin, and other antibodies in early clinical trials. Treatment with the anti-TNFα antibodies infliximab, adalimumab, and certolizumab pegol are effective for treating moderate to severe CD, with 60% initial responses and maintenance of responses for 6 – 12 months in 40%. Enteracept is not effective. Anti-TNF agents are reasonably well tolerated but carry risks of infusion reactions; antibody formation; infections caused by organisms from pyogenic foci, M. tuberculosis, and herpesviruses; and development of lymphomas. Fatal infections have been seen [35,36]. Experts in this area have the impression that aggressive treatment that includes anti-TNF therapy early in the course of CD can lead to mucosal healing without resorting to prolonged prednisone exposure. The most recent randomized trial examining optimal medical therapy for treatment of young adults with moderate to severe CD found that an infliximab/azathioprine regimen resulted in ~50% of patients being in glucocorticoid-free remission at one year [37]. Anti-α_4-integrin therapy with natalizumab, designed to inhibit leukocyte migration into inflamed gut tissues, showed evidence of efficacy in treatment and maintenance of patients with moderate to severe CD. Development of JC-virus-related progressive multifocal leukoencephalopathy in seropositive patients and rare cases of hepatic necrosis suggest that safety issues may limit this approach to long-term care in patients who are susceptible to these complications. Other antibody-based therapies in clinical trials include anti-IL12, anti-IL6, and anti-CD3.

Surgical approaches are indicated for patients with abscesses, medically-intractable fistulas, fibrotic intestinal strictures leading to obstruction, toxic megacolon, cancer, and refractoriness to medical therapy, particularly when prolonged glucocorticoid exposure and toxicity are present. Several series have noted that 25-45% of patients require surgery within 3 years of diagnosis; 25 – 38% of resected patients require a second operation within 5 years; and ~33% of these undergo a third surgery [38]. Recurrent CD almost always occurs proximal to the anastomosis. After 20 years have passed from disease onset, about 75% of patients with CD will have had a surgical procedure [39]. Surgeons strive to preserve intestine to avoid malnutrition and requirement for TPN support, in the case of short bowel syndrome.

Nutritional approaches have proven disappointing as primary treatment modalities, but in patients with protein calorie malnutrition and specific micronutrient deficiencies (e.g., fat soluble vitamins, B12, folate, calcium), supplements are critical to good health. Some patients with severe CD and many patients with short bowel syndrome require TPN to sustain life.

Novel therapies have been legion in a disease without a definitive cure. Current investigational agents include porcine whipworm (induction of T regulatory cells), GM-CSF (correcting defective innate immune responses), mesenchymal stem cells, thalidomide or lenalidomide, probiotic approaches, and approaches aimed at unique therapeutic targets (cytokines, adhesion molecules, T regulatory cells, for example) [32,40].

2.1.8. Econometric considerations in CD. While the mortality rate in patients with CD is higher than in controls in population-based studies, the fact is that this disease, with its usual onset in the teen and young adult years, permits substantial longevity, albeit with a waxing and waning of chronic illness in the majority of patients. Several econometric studies have been carried out before the era of biologic response modifier therapy, that is, patients were treated with standard medical therapies and surgery for complications. In these studies, surgical and hospitalization costs comprise the most expenditures. No published CD population-based data has been published in the current era of expensive biologic response modifier therapy, but the hypothesis is that the cost of ongoing maintenance therapy would be partially offset by avoidance of hospitalization and surgery [41]. Yearly costs of maintenance therapy plus the cost of caring for flares, multiplied by a nearly normal lifespan for the average CD patient, amounts to a considerable cumulative cost.

2.1.9. Morbidity and mortality. While there is variation from patient to patient in the natural history of CD, morbidity is highest in patients with persistent, unresponsive disease; recurrent disease after surgical or medical therapies; and major fistulizing or stenotic complications. Over time, approximately 10% of patients with CD are disabled by their disease and its many complications [33,42-50]. Although CD is usually either a chronic, unremitting illness or one that is characterized by remission/relapse cycles of activity, the actuarial mortality risk is

only 40% higher than in controls: 66.9 vs. 49.7 deaths per 10,000 person years [51]. Excess deaths are related to the disease itself, surgical complications, and cancer [35,52-58]. When CD involves the colon, the risk of colon cancer is the same as in patient with long-standing ulcerative colitis. There are also increased risks of small intestinal adenocarcinoma, anal canal squamous cell carcinoma, and possibly lymphomas. Paradoxically, the advent of more effective biologic therapies to control signs and symptoms of CD has led to fatal infections related to immune suppression [35,36].

2.2 Clinical data to date on hematopoietic cell transplantation for autoimmune diseases, aplastic anemia, and Crohn's Disease.

Data from numerous centers show that both autologous and allogeneic HCT alter the natural history of autoimmune diseases and aplastic anemia. Data from Northwestern University show that a cyclophosphamide-based conditioning regimen followed by autologous HCT results in clinically-meaningful remissions of CD, but with evidence that over five years of follow-up, the frequency of recurrent CD requiring additional treatment increases with time [59]. Data from two centers support the hypothesis that allogeneic HCT can result in sustained remissions of CD [60,61]

2.2.1. Results of autologous HCT as treatment for autoimmune diseases. High-dose immunosuppressive therapy (HDIT) followed by autologous HCT for autoimmune diseases (AID) [62-73] has been well-tolerated; significant responses have been observed with refractory severe autoimmune diseases [62-71,73,74]. Although some patients have had durable responses, in others a high-incidence of relapse has been noted, e.g., rheumatoid arthritis [59,75,76].

2.2.2. ***Results of autologous HCT as treatment for CD.*** HDIT followed by autologous HCT for CD has yielded clinically meaningful improvement in the years following transplant [59,77] [Hawkey CJ. Dig Dis 2012; 30 (suppl) 3): 139-139]. The series with the longest follow-up enrolled 24 patients (average age 27) who had failed medical and surgical therapies. A conditioning regimen of cyclophosphamide 200 mg/kg and antithymocyte globulin was used. Bacteremias and fever were common until engraftment, but importantly there were no transplant-related deaths in this cohort. A prompt fall in CD activity was seen, as reflected by CDAI scores. However, with 5 year follow-up, clinical relapse free survival (free of medical therapy) is 96% at 1 year, 63% at 3 years, and 36% at 5 years [59]. Eight patients required surgical resections for recurrent CD. Among patients requiring immunosuppression to sustain clinical CD remission after autologous transplant, immune tolerance was not established and it is expected that most autografted patients will relapse with further follow-up, given the strong genetic influence for CD [78,79]. A multicenter, randomized trial of autologous HCT vs. delayed autologous HCT for CD (the Autologous Stem Cell Transplantation International Crohn's Disease – ASTIC – trial) has reported interim results.[Hawkey C et al. Clinical and endoscopic improvement following hemopoietic stem cell transplantation vs. mobilization alone in Crohn's Disease. Gut 2013; 62 (Suppl 1): A6 (OC-014)]. As of January 2013, data from 34 patients were available, showing that autologous transplant can substantially reduce clinical severity and endoscopic appearance of CD but with significant toxicity, including one transplant-related death.

2.2.3. Results of allogeneic HCT as treatment for aplastic anemia and autoimmune diseases. Allogeneic HCT from an HLA-matched donor is a standard therapy for aplastic anemia. In a report of 94 patients, overall survival was 88% with a median follow-up of 6 years and all mortality events occurred before 1 year after HCT [80]. Grade II and grade III-IV acute GVHD was observed in 21% and 8% of patients respectively. Chronic GVHD occurred in 32% and most responded completely to treatment. At a median of 2.6 years after allogeneic HCT, only 8% of patients required immunosuppressive therapy. Data from our center suggests that limiting the marrow cell dose was associated with a reduced incidence of chronic GVHD [81]. In a follow-up prospective study of 14 patients with aplastic anemia, we found that when the marrow cell dose was limited to 2.0-2.5 × 10^8 nucleated cells/kg, no cases of grade III-IV acute GVHD occurred, only 1 patient has developed chronic GVHD, and there have been no deaths during a median follow-up of 2 years (unpublished). Outcomes have improved for allogeneic HCT of aplastic anemia and similar or better outcomes may be possible after allogeneic HCT in patients with CD since patients will not be pancytopenic before HCT, although they may be immune suppressed.

There have been numerous publications showing that allogeneic HCT may induce sustained remissions of autoimmune diseases (AID) (reviewed in [82]). In a series of patients with blood disorders and a concomitant AID, freedom from relapse of the AID was 89% at 18 years after allogeneic HCT vs. 38% at 5 years after autologous HCT (p= 0.0002) [83].

2.2.4. Results of allogeneic HCT as treatment for patients with primary immune deficiency disorders who have underlying autoimmune enteropathies similar in phenotype to CD. Several studies have shown

excellent outcomes with disease resolution following allogeneic HCT using both related and unrelated donors in patients with primary immune deficiency disorders who have underlying autoimmune enteropathies similar to patients with CD. Patients with IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) often have significant diarrhea to the point of TPN dependence due to significant failure to thrive. Allogeneic HCT has resulted in resolution of their underlying enteropathy [84-86]. In addition, several studies have shown disease resolution following allogeneic HCT for patients with a rare immune deficiency resulting from a mutation in the Interleukin-10 receptor. As a result , patient's cells are unresponsive to the anti-inflammatory cytokine IL-10. This immune deficiency is characterized by a severe, early onset, fistulating colitis for which bone marrow transplantation is the only therapy that offers any significant benefit [87,88]. Available medical therapy for this condition is limited to supportive care and if patients do not receive allogenic HCT, patients typically die of severe, uncontrolled bowel inflammation.

2.2.5. ***Results of allogeneic HCT as treatment for CD.*** When patients with CD who underwent allogeneic HCT for leukemia were studied, those who achieved donor chimerism had resolution of signs and symptoms of CD that was sustained for up to 15 years following HCT. In patients with leukemia and concomitant CD, we reported in 1998 that 4 of 5 evaluable patients had no signs of CD after allogeneic HCT with a median follow-up of 8.4 years [60]. These observations were later confirmed in a similar series in which 11 patients with inflammatory bowel disease (CD=7; ulcerative colitis=4) and leukemia underwent allogeneic HCT. Of 10 surviving patients, none showed IBD activity at a median of 34 months after allogeneic HCT except for 1 patient with mild persistent symptoms after a limited follow-up [61]. Rare autosomal recessive mutations in the genes encoding IL-10 and the IL-10 receptor lead to a clinical disease indistinguishable from CD; children with these mutations have been cured with allogeneic HCT [87,88]. These case series suggest that allogeneic HCT has substantial potential to cure CD.

2.3 Study Regimen

After first screening, it may take several months to obtain approval by third-party payers for transplant and find a suitable HLA-matched donor. Since patients being considered for this protocol will have treatment-refractory active CD, it is possible that intestinal ulcerations, fistulas, phlegmons, and abscesses will require treatment before eligibility criteria are met. Medical and possibly surgical therapy may be required, at the direction of each patient's referring physician. It is anticipated that therapies such as methotrexate, monthly pulse cyclophosphamide, and thiopurine antimetabolites might be used to achieve short–term improvements.

For patients who meet eligibility criteria, we propose to use a reduced-intensity conditioning regimen of fludarabine, cyclophosphamide , and low dose total body irradiation (FLU-CY-TBI) which has been successfully piloted for transplantation of marrow grafts from HLA-haploidentical donors [89]), a regimen that has resulted in successful outcomes despite past difficulties with engraftment and GVHD after haploidentical transplants. A reduced-intensity regimen was chosen over a myeloablataive regimen to decrease the risk of severe regimen-related toxicities and thus, decrease transplant-related mortality in patients with refractory CD. To prevent acute and chronic GVHD, high-dose cyclophosphamide will be given on day +3 and +4, along with tacrolimus and Myfortic starting on day +5. To further reduce the risk of GVHD, related and unrelated donors will be required to be HLA-matched and the source of the graft will be marrow rather than peripheral blood. Supportive care will include N-acetyl cysteine infusions to restore hepatocyte and sinusoidal endothelial cell levels of reduced glutathione (GSH) to reduce the risk of sinusoidal liver injury [90]; prophylaxis with ursodiol, started two weeks before the start of conditioning therapy (to prevent cholestatic liver disease) [91,92]; and antimicrobial drugs as prophylaxis and pre-emptive treatment for infections caused by bacteria, fungi, herpesviruses, and Pneumocystis jiroveci.

Engraftment of allogeneic marrow grafts has been successfully achieved after both standard-dose and reduced-intensity conditioning regimens. To reduce regimen-related toxicity in patients already suffering from injury to the gastrointestinal tract, a reduced-intensity conditioning regimen of low-dose cyclophosphamide (50 mg/kg), fludarabine (150 mg/m^2) and low-dose TBI (200 cGy) will be used in this study.

Importantly, this FLU-CY-TBI regimen has been used effectively to achieve engraftment of allogeneic hematopoietic cell grafts from HLA-haploidentical donors [89,93,94]. In these reports of 66 patients, graft rejection occurred in 9 recipients [89]. All but 1 of the patients experiencing graft rejection had recovery of autologous hematopoiesis. If graft rejection had occurred after a myeloablative conditioning regimen, autologous recovery would have been expected to have been very delayed or not to have occurred. In a more recent phase 2 study by BMT-CTN investigators of reduced-intensity conditioning and marrow transplantation from HLA-haploidentical donors followed by high-dose cyclophosphamide (n=55), only one patient experienced graft failure [95,96]. The barrier to engraftment is greater with marrow grafts from HLA-haploidentical donors than it would be from HLA-

matched donors; thus, the risk of graft rejection is expected to be less with allografts from the HLA-identical donors in this protocol. The protocol includes the collection of autologous cells that will be held in reserve for use in the event of graft rejection.

For patients conditioned with a myeloablative regimen of busulfan and cyclophosphamide and transplanted with cells from HLA-matched related or unrelated donors, post-transplant high-dose cyclophosphamide has been studied for GVHD prevention [34]. In these studies, a myeloablative conditioning regimen was used because of concern about the increased risk of relapse of hematological malignancies resulting from the potentially reduced graft-vs-leukemia effect associated with the expected reduced incidence of GVHD from post-transplant cyclophosphamide infusions. However, recurrent malignancy is not a concern in patients with CD and there is likely little benefit derived from a myeloablative conditioning regimen, which increases the risk of severe regimen-related toxicity. Therefore, there is rationale for using reduced intensity conditioning in high-risk patients with CD. Multiple studies have shown excellent outcomes following reduced-intensity conditioning followed by HLA-matched related or unrelated grafts for patients with nonmalignant diseases [97].

The major risk associated with allogeneic HCT is acute and chronic GVHD. High-dose cyclophosphamide at day +3 and +4 in combination with tacrolimus and MMF has been effective for reducing the risk of GVHD after transplantation from HLA-haploidentical donors and was associated with low transplant-related mortality [89]. In this report, the cumulative incidences of grades II-IV and grades III-IV acute GVHD by day 200 were 34% and 6%, respectively. In the recent study from BMT-CTN of haploidentical grafts following the FLU-CY-TBI regimen with posttransplant cyclophosphamide and tacrolimus/MMF for GVHD prevention, the incidence of grade II acute GVHD was 31% at day +56 and no grade III-IV acute GVHD was reported [95,96]. The incidence of chronic GVHD at day +180 was 11%. In the report by Luznick et al of a phase 2 study on the use of high-dose cyclophosphamide alone after marrow transplantation from HLA-matched related and unrelated donors, the incidence of grade II-IV and grade III-IV acute GVHD was 43% and 10%, respectively [34]. Patients received posttransplant high-dose cyclophosphamide without tacrolimus or MMF for prevention of GVHD. Since patients with nonmalignant disorders such as CD do not benefit from GVHD, we propose to give patients tacrolimus and MMF in addition to the high-dose cyclophosphamide to reduce the incidence of GVHD. It is expected that the increased posttransplant immunosuppression following the reduced – intensity FLU-CY-TBI regimen would also result in a decreased risk of graft rejection. High-dose cyclophosphamide posttransplant in combination with tacrolimus and MMF has been well-tolerated after transplantation from HLA-haploidentical donors with a TRM of 5% at 6 months and 7% at 12 months [95,96]. TRM may be less after transplantation from HLA-matched donors since GVHD should occur less frequently and the use of high-dose corticosteroids will be less.

In summary, the proposed reduced-intensity conditioning regimen for this study is expected to be safe in patients with CD and to be sufficiently immunosuppressive to prevent graft rejection after transplantation of marrow grafts from HLA-matched donors. Post-transplant cyclophosphamide in combination with tacrolimus and intravenous MMF or oral enteric coated mycophenolic acid (Myfortic) is expected to reduce substantially the risk of GVHD and to improve overall outcomes after transplant. Myfortic will be preferentially used instead of MMF when oral medications are tolerated because it is associated with significantly fewer gastrointestinal adverse effects than MMF.

2.4 Rationale for the proposed plan of treatment

Conventional immunosuppressive or immunomodulatory therapy may not be adequate for achieving and maintaining remission in patients with severe CD. These patients experience ongoing medical problems and frequent surgeries putting them at risk for short bowel syndrome. High-dose immunosuppressive therapy followed by autologous HCT results in effective early control of CD but most patients relapse by 5 years and, based on the slope of the event-free survival curve, all patients would be expected to relapse if followed long enough [59]. The high relapse rate after high-dose immunosuppressive therapy followed by autologous HCT might be expected since there is a strong genetic factor predisposing patients to CD. We have proposed to investigate treatment of CD with allogeneic HCT since sustained remissions have been observed in CD patients transplanted for hematological malignancies [60,61].

2.5 Risks/Benefits

2.5.1 Risk of death and disability as a result of allogeneic hematopoietic cell transplantation

The causes of death after allogeneic HCT can be broadly categorized as follows: 1) toxicity from the conditioning regimen that affects the liver, lungs, heart, kidneys, and gastrointestinal tract; 2) infections during times of cellular

and humoral immune deficiency; 3) acute GVHD; 4) bleeding as a result of low platelet counts; 5) complications related to medications; and 6) chronic GVHD. To increase safety of the procedure, a reduced-intensity conditioning regimen will be used to decrease severe regimen-related toxicity and related infections. There is a risk of graft rejection following reduced intensity conditioning regimen in patients who receive HLA matched grafts. Unpublished data was forwarded from Ephraim Fuchs and Carol Ann Huff on the Johns Hopkins University experience with a dose escalation trial for patients with hematologic malignancy based on a conditioning regimen of fludarabine (30 mg/m^2/day x 3 days) plus 200 cGy TBI on day -1, followed by marrow or PBSC allograft from an HLA-matched donor and post-transplant cyclophosphamide. The groups and graft outcomes were as follows:

Arm A – CY 50 mg/kg on day +3 only (2/4 engrafted)
Arm B – CY 50 mg/kg on days +3 and +4 only (5 /8 engrafted)
Arm C – CY 50 mg/kg on days +3 and +4 and MMF 15 mg/kg BID days 5-34 (21/24 engrafted)
Arm D – CY 50 mg/kg days +3 and +4 and MMF 15 mg/kg BID with peripheral blood graft (28/30 engrafted).

The data suggest that post-transplant high-dose cyclophosphamide does not increase the risk of graft rejection and that the addition of MMF to the post-transplant regimen decreases the risk of graft rejection. The conditioning regimen for this protocol will be more immunosuppressive than that used at Hopkins and includes Flu 150 mg/m^2 over 5 days, 50 mg/kg of cyclophosphamide and TBI (200 cGy). Post-transplant, patients will be immunosuppressed with both tacrolimus BID and Myfortic TID in the Crohn's protocol whereas JHU protocol patients received only MMF BID in groups 3 and 4. That is, as the proposed conditioning and immune suppression in this protocol is substantially more immune suppressive than what the JHU group gave to patients in their Arm C, the increased intensity of the pre-transplant conditioning and the addition of both tacrolimus and Myfortic post-transplant is expected to reduce the risk of graft rejection in the proposed transplant regimen for Crohn's disease. To reduce the risk of a fatal outcome in the event of graft rejection and pancytopenia, autologous stem cells will be harvested and stored for use if such an eventuality occurs. The risk of infection will be reduced by use of prophylactic and pre-emptive therapies for microbes. After transplant, treatment with high-dose cyclophosphamide, tacrolimus, and Myfortic will reduce the risk of acute GVHD. In a recent report of a multicenter study, transplant-related mortality was 7% at 12 months after transplantation from HLA-haploidentical donors, a type of transplant that poses more problems than we expect with HLA-matched donors. Long-term disability after allogeneic HCT is related to chronic GVHD and the protracted use of glucocorticoids. High-dose cyclophosphamide after transplant has reduced this risk but the incidence of severe disability in long-term transplant survivors may be 5% or higher.

2.5.2 Probability of a 'cure' of CD

Based on studies of CD patients undergoing allogeneic HCT for hematological malignancies, we expect that patients will likely achieve a sustained remission of CD.

2.5.3 Overview of risks and benefits

In this phase 2 study, treatment-refractory CD would be the only indication for allogeneic HCT. We considered this approach 12 years ago, following the publication of our case series showing that allogeneic HCT could effect sustained remissions of CD [60]. At that time, we estimated the mortality from allogeneic transplantation for CD at ~20%, which we thought was unacceptably high. Recent advances in the technique of allogeneic HCT have substantially reduced the mortality risk, particularly for patients with non-malignant conditions [80,81,92,95,96].

We consider these to be the mutually exclusive outcomes of patients with treatment-refractory CD:

a) Continued disability and low quality of life with no effective CD treatment, including the possibility of requiring total parenteral nutrition to sustain life;
b) A risk of death caused by cancer or a complication of surgery or related to an infection because of CD therapies that suppress the immune system;
c) Small bowel transplantation and life-long high-dose immune suppression;
d) The future discovery of a new medical therapy for CD that results in sustained disease remission;
e) Spontaneous remission of CD;
f) Sustained remissions of CD after successful allogeneic transplantation;
g) Recurrent CD after allogeneic transplantation
h) The risk of death as a complication of allogeneic transplantation.

Outcomes (a), (b), or (c) are the most likely outcomes for patients who meet eligibility criteria for this study but who are not transplanted. While outcome (d), an effective medical therapy to be discovered sometime in the future, is a possibility, it seems unlikely that any medicinal therapy will be curative for a disorder rooted in genetic susceptibility to disease. Similarly, a spontaneous CD remission is an unlikely event in patients who have already evinced progressive and debilitating disease. These adverse outcomes have to be balanced against the

possibility of a cure or long-term remission of CD following allogeneic HCT (f), while taking into consideration the possibility of recurrent CD after HCT (g), or the risk of death as a result of HCT (h).

The expected mortality following allogeneic transplantation of carefully selected patients with refractory Crohn's Disease enrolled on this protocol is zero. This figure is based on data from the Fred Hutchinson Cancer Research Center where these transplants will be carried out, specifically the outcomes of allogeneic transplantation in the 17 consecutive, most recent patients with aplastic anemia, in whom there were no deaths. Patients with aplastic anemia are an appropriate comparison cohort for those with Crohn's Disease in our protocol: Both are benign, non-malignant disorders; both are commonly treated with immune suppressive drugs; and both can be potentially cured with allogeneic transplantation. We also note that there were no deaths in the cohort of 24 patients with severe Crohn's Disease reported from Northwestern who underwent conditioning therapy with high-dose cyclophosphamide followed by autologous cell infusion [59]. For comparison, the actuarial mortality risk among all patients with Crohn's Disease (CD) is 40% higher than in controls: 66.9 vs. 49.7 deaths per 10,000 person years. Excess deaths are related to the disease itself, surgical complications, and cancer. When CD involves the colon, the risk of colon cancer is the same as in patient with long-standing ulcerative colitis. There are also increased risks of small intestinal adenocarcinoma, anal canal squamous cell carcinoma, and possibly lymphomas. Paradoxically, the advent of more effective biologic therapies to control signs and symptoms of CD has led to fatal infections related to immune suppression. Morbidity and mortality are highest in patients with persistent, unresponsive CD; recurrent CD after surgical or medical therapies; and major fistulizing or stenotic complications. Over time, approximately 10% of patients with CD are disabled by their disease and its many complications--these are the patients that we intend to enroll in this protocol. We estimate that the actuarial mortality risk is higher among patients in this 10 percentile with refractory CD than among those with responsive CD. In absolute terms, patients with refractory Crohn's Disease have an estimated 0.007 deaths per patient year, or a 0.7% risk.

Another source of data on the mortality of allogeneic transplantation is the recent experience from Johns Hopkins that demonstrated that a patient population which in the past has been very difficult to successfully allograft (patients with malignancy who received haploidentical donor cells) can now undergo successful allogeneic transplantation using improved transplant methods. In the Hopkins experience with these difficult patients, transplant-related mortality was 5% at 6 months and 7% at 12 months. We do not expect that the mortality risk in our eligible patients with Crohn's Disease will approach these figures because a) our allogeneic donors will be HLA-matched, not haploidentical; b) our patients will not have been pre-treated with chemotherapy for cancer; and c) our patients will not have a need to minimize post-transplant immune suppression to promote graft-vs-leukemia effects. In our proposed protocol, specific techniques will be employed to lower the risk of fatal complications, namely exclusion of patients with medical co-morbidities; optimal GVHD prophylaxis (we do not have to be concerned about the necessity of a graft-vs.-leukemia effect, as we would in a patient with malignancy); optimal infection prophylaxis; and the storage of autologous hematopoietic cells before the start of conditioning therapy, to be reinfused in the event of graft-rejection after transplant.

The ethical equipoise in our proposed protocol is that of a life of continued misery and disability from CD, in addition to an increased mortality risk related to CD and its complications, balanced against an expected mortality risk of zero following allogeneic bone marrow transplantation and a potential cure of CD. Published experience from our group and from investigators in Germany strongly suggest that allogeneic transplantation can cure patients with Crohn's Disease, when patients came to transplant with both a hematologic malignancy and CD. We do not use the word "cure" lightly here, but with abundant evidence pointing to underlying genetic polymorphisms as the root cause of Crohn's Disease, the replacement of a patient's dysfunctional immunogenetics with a normal immune system leads to the logical expectation of cure.

3.0 STUDY OBJECTIVES

3.1 Hypothesis

Allogeneic hematopoietic cell transplantation (HCT) can achieve complete, sustained remissions in patients with refractory CD, and can be done safely.

3.2 Primary Objectives

The primary objective is to evaluate the safety and efficacy of allogeneic HCT as treatment for refractory CD.

3.3 Secondary Objectives

To evaluate treatment effect on CD activity and severity.

To evaluate safety of allogeneic HCT as determined by regimen-related toxicities, infectious complications, acute and chronic GVHD, treatment-related mortality, overall total mortality, and time to engraftment.

To evaluate the effect of allogeneic HCT on quality of life (QOL) in patients with severe refractory CD.

4.0 STUDY DESIGN

4.1 Description of Study

This study is a prospective, open-label, single-arm Phase II clinical trial evaluating allogeneic HCT for the treatment of refractory CD. Eligible subjects will have active CD that has responded poorly to standard therapy and for whom an eligible donor has been identified; a description of each patient's CD history will be recorded (Appendix D). Eligible donors will be an HLA genotypically identical sibling or an unrelated donor who is HLA-matched by standard molecular methods. Autologous hematopoietic cells will be collected and stored, to be used in the event of graft rejection after transplant. Patients will receive a reduced intensity conditioning regimen of cyclophosphamide, fludarabine and low-dose TBI. Marrow will be used as the graft source instead of peripheral blood stem cells to reduce the risk of GVHD. GVHD prophylaxis will consist of post-transplant high-dose cyclophosphamide followed by the combination of tacrolimus and enteric coated mycophenolic acid.

Subjects will be extensively studied for safety and efficacy utilizing clinical assessments, laboratory testing, endoscopy of the gastrointestinal tract, and histologic examination of tissue samples. Tissue and blood samples will be collected and archived for future studies and evaluation of immune reconstitution at predefined intervals. Well-validated scoring systems will be used at baseline and at intervals after transplant, specifically for activity of CD (CDAI), quality of life (SIBDQ), and endoscopic extent of inflammatory lesions (SES-CI), shown in Appendices A, B, and C, respectively.

This study will enroll 12 subjects over a 2-year period. The active treatment period will be approximately 3-4 months after transplant (day 0). Evaluations of CD status and safety endpoints are at 1 month, 3 months, and 12 months then annually up to 60 months (5 years). Subjects are expected to be in study for 5 years. Total study duration will be 84 months (7 years) from transplant of first patient to the 5-year evaluation of the last subject who was transplanted.

4.2 Endpoints

Primary Endpoint

The primary endpoint will be event-free survival at 1 year after transplant, defined as alive and free of active CD. Active CD is defined as abnormal mucosal inflammation characteristic of CD identified by endoscopy and biopsy of the gastrointestinal tract. Event-free survival has been selected as the primary endpoint measurement because it permits the evaluation of both efficacy and safety.

Secondary Endpoints

1. Event-Free Survival - The EFS from 2 through 5 years will be calculated from the time of treatment. An event is defined as for the primary endpoint. Patients alive at the time of last contact are censored.

2. Overall Survival - Overall survival at 1 through 5 years will be calculated from the time of treatment assignment. Death due to any cause is the event for this endpoint. Patients alive at the time of last contact are censored.

3. Treatment-related mortality - TRM is defined as death occurring at any time after start of allogeneic HCT and definitely or probably resulting from treatment given in the study and not associated with other unrelated causes. The protocol team will attribute cause of death after reviewing clinical and autopsy data in order to determine this endpoint.

4. Regimen-related Toxicity - Adverse events (AEs) as defined by the modified Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 and as described in section 13.2 and Appendix J. AEs will be tabulated for each patient; reporting requirements for AEs and SAEs in patient enrolled on this protocol are described in Appendix J.

5. Development of Infectious Complications - The incidence of definite and probable viral, fungal and bacterial infections will be tabulated for each patient.

6. Quality of Life – will be measured using the previously validated Short Inflammatory Bowel Disease Questionnaire for adults.

7. Disease activity- Activity will be evaluated using a standardized tool for evaluating CD (Crohn's Disease Activity Index (CDAI) for adults.

8. Incidence of Graft Rejection- Engraftment is defined as achieving >5% donor peripheral blood CD3 T cell chimerism by Day 84 after HCT. Primary graft failure is defined as a donor peripheral blood CD3 T cell chimerism peak of <5% by Day 84 post-HCT. Secondary Graft Failure is defined as documented engraftment followed by loss of the graft with donor peripheral blood CD3 T cell chimerism <5% as demonstrated by a chimerism assay.

9. Incidence and Severity of Graft versus Host Disease - The grading of acute and chronic GVHD will follow previously published guidelines but will also include capture of symptoms and characterization of alternative causes (Appendices F and G). The highest level of organ abnormalities, the etiologies contributing to the abnormalities and biopsy results pertaining to GVHD will be identified. Since both GVHD and CD involve the gastrointestinal tract, all diagnostic biopsies of these organs will be reviewed by pathologists experienced in the diagnosis of GVHD and IBD, respectively.

10. Incidence of Disease-Modifying Drugs for CD Initiated Post Transplant. Subjects are not expected to receive additional disease-modifying therapy for CD in the absence of disease activity, as defined above. A decision to initiate disease-modifying therapy other than that specified in the protocol after allogeneic HCT will be considered treatment failure and thus, fulfill a secondary endpoint. In general, this would include the administration of any therapy (drugs, biologics, or any other treatments) clearly given as immunomodulatory therapy for underlying CD. Use of immunosuppressive agents for the treatment for GVHD in the transplant arm will not be considered for this endpoint.

5.0 SUBJECT SELECTION

Potential patients will be referred to the study by gastroenterologists or be self-referred. Potential patients will have access to information regarding the clinical trial and can provide information to determine if they are eligible for the study. No further follow-up of these patients will be undertaken if they are ineligible. If patients are potentially eligible, then this information will be forwarded to the study team and follow-up will be done by the study coordinator. The study team may also be contacted directly by the patient or the patient's physician by telephone or by email.

If the patient is potentially eligible for study, the study team will request medical records that will be reviewed by the study coordinator and P.I. If, after this assessment, the patient is still potentially eligible for this protocol, the patient will be invited to Seattle for further evaluation at a **First Phase Screening Visit** (see section 11.1). An informed consent document for the detailed screening by a study investigator will be completed before the evaluation in Seattle. Patients will be counseled regarding the experimental nature of allogeneic HCT for CD. After the screening, an eligibility checklist (Appendix E) will be completed and forwarded to the Eligibility Review Group, composed of one gastroenterology investigator, one transplant investigator, and the PI (see list of investigators, page 1 of this protocol). This group must approve all transplants for this protocol.

Following approval by the Eligibility Review Group, medical insurance companies or third-party payers will then be contacted to request coverage for the donor search and the procedure of allogeneic hematopoietic cell transplantation, as described in this protocol. After confirmation of coverage for these procedures is obtained, HLA typing and a donor search will be started. Patients with HLA-matched related or unrelated donors will return to Seattle for a **Second Phase Screening Visit** (see Section 11.2) consisting of baseline studies including additional disease staging by gastroenterologists on the study team. The informed consent document for

autologous peripheral blood stem cell collection and hematopoietic cell transplantation will be signed after baseline studies are completed and the patient is counseled in depth about the protocol by a transplant attending physician (**Pre-transplant counseling, testing, and informed consent**, section 11.3).

5.1 Inclusion Criteria

CD is an inflammatory, often treatment-refractory disease that is evaluable using imaging and endoscopic means. Eligible patients would be those who continued to have significant symptoms or disruption of quality of life (as defined by CDAI and SIBDQ, respectively) caused by intestinal inflammation despite standard immune suppressive therapies (including anti-TNFα) and surgical approaches to strictures and fistulae, or who were facing small intestinal resection that would result in short bowel syndrome.

1. A diagnosis of CD established by referring physician(s) and confirmed by our review of the clinical presentation, clinical course, endoscopic and imaging findings, and histology of mucosal tissue specimens.

2. An adverse prognosis, documented by persistent signs and symptoms of CD that have failed to respond satisfactorily to medical and surgical therapies in the past, including but not limited to systemic immune suppressive drugs and biopharmaceuticals, as outlined above in section 2.1.6. To be considered as refractory to medical and surgical therapy, there must be clinical, endoscopic, and histologic evidence of active inflammatory Crohn's Disease that has either persisted or recurred despite exhaustive treatment with available pharmaceutical and surgical therapies. Exhaustive treatment will be defined as prior exposure to the following, without durable improvement:

   a) Systemic glucocorticoids at or above a prednisone equivalent of 40 mg/day for at least 2 weeks, or until drug toxicity or intolerance develops.

   b) Methotrexate (25 mg per week for at least 3 months, or until drug toxicity or intolerance develops) and/or a thiopurine antimetabolite (either 2.5 mg/kg azathioprine or 1.5 mg/kg 6-mercaptopurine in patients homozygous wild-type for the TPMT gene, or either 1.5 mg/kg azathioprine or 1 mg/kg 6-mercaptopurine in patients heterozygous for TPMT, or doses of these drugs capable of producing a 6-thioguanine nucleotide level of 230-400 without producing a 6-methylmercaptopurine nucleotide level above 5700 for at least 3 months, or until drug allergy, intolerance or toxicity develops). If a patient is homozygous mutant for the TPMT gene, thiopurines would be contraindicated and their use would not be a requirement for enrollment in this protocol.

   c) Use of at least two anti-TNF-alpha therapies, that is, infliximab (at least 5 mg/kg every 8 weeks for at least 3 months, or until drug allergy, toxicity or intolerance or anti-infliximab antibodies develop) and/or adalimumab (at least 40 mg SQ every 2 weeks for at least 3 months, or until drug allergy, toxicity or intolerance develops) and/or certolizumab pegol (at least 400 mg SQ every 4 weeks for at least 3 months, or until drug allergy, toxicity or intolerance develops).

   d) Due to the serious risk of Progressive Multifocal Leukokencephalopathy (PML) and the reluctance of some patients to agree to therapy that carries such risk, prior exposure to natalizumab is not required to meet the definition of exhaustive pharmaceutical treatment. Neither will use of natalizumab among patients who are JC virus antibody seronegative be an exclusionary criterion.

   e) Exhaustive surgical treatment will be defined as indicated operations for complications of Crohn's Disease up to the point where the risks of surgery (for example, mortality or post-operative morbidity such as short bowel syndrome or extensive adhesions with high risk for inadvertent enterotomy) are deemed by patients and their physicians to be unacceptably high. Indicated operations for complications of Crohn's Disease include, but are not limited to, surgical resection of involved intestine, stricturoplasty, drainage, curettage, or adhesiolysis of tissues affected by Crohn's disease).

   f) Exposure of patients to investigational drug therapies for Crohn's Disease, that is, to drugs that are not FDA approved for this indication, will not be a criterion for either inclusion or exclusion.

   The intent of these requirements is to identify patients with Crohn's Disease whose signs, symptoms,

and intestinal inflammation have not been controlled by the best available therapies and whose future is highly likely to be one characterized by disability and misery.

3. Endoscopic and histologic evidence of active intestinal inflammation consistent with CD. In the event that the involved mucosa cannot be readily reached by endoscopic biopsy, an imaging test that shows typical changes of CD in the intestinal tract will suffice as evidence of active intestinal inflammation. The presence of intestinal stomas does not exclude the patient from study.

4. Severe CD as defined by one of the following:
    a. CDAI ≥250
    b. Need for total parenteral nutrition to maintain weight
    c. Recurrent intestinal inflammation caused by CD following surgical resection

5. Identification of an HLA-matched hematopoietic cell donor without a history of a disorder that can be transmitted by hematopoietic cells, including but not limited to inflammatory bowel disease, and without NOD2 mutations in the case of an HLA matched sibling.

6. Age from 18 through 60 years.

5.2 Exclusion Criteria

1. A current complication of CD that would jeopardize survival after hematopoietic cell transplantation, including but not limited to the following:
    a. Abscess, phlegmon, necrotizing skin lesion, or inflammatory fistula
    b. Intestinal fibrotic stricture and intestinal obstruction
    c. Uncontrolled mucosal, organ, or systemic infection with a bacterial, viral, fungal, or parasitic organism
    d. Sclerosing cholangitis

2. History of Progressive Multifocal Leukoencephalopathy

3. Organ dysfunction or disease that would jeopardize survival after hematopoietic cell transplantation, including but not limited to the following:
    a. Renal insufficiency as defined by an estimated GFR < 60 mL/minute
    b. Cardiac dysfunction as defined by symptomatic coronary artery disease, congestive heart failure, valvular heart disease, cardiomyopathy, uncontrolled arrhythmia(s), or left ventricular ejection fraction <50%
    c. Pulmonary dysfunction that poses a risk of mortality after transplant, as defined in Appendix F as Pulmonary disease-moderate, using pre-transplant pulmonary function testing per the FHCRC Standard Practice Manual.
    d. Necroinflammatory or fibrotic liver disease with evidence of liver dysfunction, including but not limited to jaundice, hepatic encephalopathy, or portal hypertension
    e. Marrow dysfunction that poses a risk of peri-transplant mortality, defined as an absolute neutrophil count or lymphocyte count below the lower limit of normal, or a platelet count below 50,000/mm^3.
    e. Poorly controlled hypertension despite appropriate therapy, defined as a diastolic blood pressure greater than 90 mm Hg while on therapy.
    f. Neurologic dysfunction that affects activities of daily living and medical care
    g. Poorly controlled diabetes mellitus, defined as persistent hyperglycemia despite therapy or recurrent hypoglycemia while on therapy.
    h. Extreme protein-calorie malnutrition defined by Body Mass Index <18 kg/m^2 and unintentional weight loss (3 kg in the last month or 6 kg in the last 6 months [98,99].

4. Pregnancy
5. Fertile men or women unwilling to use contraceptive techniques during and for 12 months following transplant
6. History of smoking either tobacco or other herbal products in the last 3 months.
7. HIV, HBV, or HCV seropositivity
8. Patients whose life expectancy is severely limited by illness other than CD
9. Untreated psychiatric illness, including drug/alcohol abuse, that would compromise compliance
10. Inability to give voluntary informed consent or obtain a parent or guardian's informed consent
11. Demonstrated lack of compliance with prior medical care

12. History of a malignancy, excluding adequately treated squamous cell skin cancer, basal cell carcinoma, and carcinoma in situ
13. Hematopoietic Cell Transplant-Co-morbidity Index greater than 2 (Appendix F)

6.0 DONOR SELECTION

6.1 Inclusions

1. Donors will be an HLA-identical sibling or HLA-matched unrelated donor.

   Unrelated donors are required to be matched by high resolution allele level typing for HLA-A, B, C and DRB1 and intermediate resolution SSOP, identifying alleles in groups of related families historically defined as antigens for DQB1. An unrelated donor is considered matched if patient and donor share HLA-A, B, C alleles with identical sequences at exons 2 and 3, DRB1 alleles with identical sequences at exon 2, and DQB1 results that include the same allele groups.

2. Donors will have the ability to understand and the willingness to sign a written informed consent document for bone marrow harvest.

6.2 Exclusions

1. Identical twin
2. Pregnant or lactating females
3. HIV seropositivity or presence of HBV DNA or HCV RNA in the serum
4. Current serious systemic illness including uncontrolled infections
5. Malignancy within 10 years prior to donation of marrow, excluding adequately treated squamous cell skin cancer and basal cell carcinoma. Treatment must have been completed (with the exception of hormonal therapy for breast cancer) with cure/remission status verified for at least 10 years at time of marrow harvest.
6. History of or symptoms consistent with inflammatory bowel disease or a serious autoimmune disorder.
7. Homozygous NOD2 mutation
8. History of a serious disease or disorder that could be adoptively transferred by infusion of donor hematopoietic cells.
9. Failure to meet institutional criteria for donation as described in the Standard Practice Guidelines

7.0 INFORMED CONSENT OF SUBJECT AND DONOR

Subjects will be referred here for consideration of an allogeneic marrow transplant. Both subject and donor will be completely evaluated. The protocol will be discussed thoroughly with subject, donor and family, and all known risks to the subject and donor will be described. The procedure and alternative forms of therapy will be presented as objectively as possible and the risks and hazards of the procedure explained to the subject or, in the case of minors, to the subject's responsible family members. Consent will be obtained using forms approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center. A summary of the conference will be dictated for the medical record detailing what was covered.

8.0 SUBJECT REGISTRATION

Eligible subjects will be identified and registered into the system by the Clinical Coordinators Office (CCO) (Intake Office) and assigned a UPN (Unique Patient Number). The CCO will register the subject on to the protocol through the Data Management Office.

9.0 SUBJECT WITHDRAWAL

9.1 Description of Subject Completion

Subjects are considered to have completed the study if they have undergone transplantation and have completed 5 years of follow-up.

9.2 Withdrawal of Individual Subjects

The investigator(s) will make every reasonable effort to keep each subject in the study through the year-5 visit. If a subject withdraws from the study, the reason for withdrawal must be documented in the study chart. Possible reasons for withdrawal include:

- Patient's preference
- Adverse experiences
- Protocol deviation, including non-compliance
- Subject lost to follow-up
- Discretion of investigator(s)

Subjects shall be withdrawn from the study immediately if any of the following occur:

- The subject or subject's guardian requests withdrawal from the study.
- The investigator believes it is in the best interest of the subject.
- There is clinically significant deterioration of the subject's medical status that warrants termination from the study.
- There are clinically significant abnormal laboratory results that warrant termination from the study.

9.3 Withdrawal of Subject from Study Following Adverse Events

The site investigator must apply his/her clinical judgment to determine if an adverse event (AE) is of sufficient severity to require that the subject should immediately be withdrawn from the study. If the withdrawal from the study is due to an AE, the subject should be given appropriate care under medical supervision until the symptoms of the AE resolve or his/her condition becomes stable. Subsequent review by the DSMB, the FHCRC IRB or the Steering Committee may also result in the suspension of further trial treatments at a site. The DSMB retains the authority to suspend additional enrollment and treatments for the entire study as applicable.

A subject may also voluntarily withdraw from treatment. If voluntary withdrawal is requested, or if withdrawal occurs for any reason, the subject should be asked to continue (at least limited) scheduled evaluations, complete an end-of-study evaluation, and be given appropriate care under medical supervision until the symptoms of any AE resolve or the subject's condition becomes stable.

9.4 Procedures for Handling Withdrawals

In case of early withdrawal, subjects should be asked to continue (at least limited) scheduled evaluations and to complete an end-of study evaluation which includes all scheduled exams, procedures, and laboratory tests as if it is the year-5 visit; and should be given appropriate care under medical supervision until the symptoms of any AE resolve or the subject's condition becomes stable. If the subject is unwilling or unable to complete visits, he/she should be asked to return for the end-of-study visit and then should be followed through telephone calls by the site study coordinator.

Subjects who do not complete study therapy will continue to be followed to the extent possible and asked to provide the same data as subjects who did not withdraw.

9.5 Subject Replacement

Subjects will not be replaced once treated with allogeneic transplantion. Subjects will be encouraged to continue in more limited follow-up even if they drop out.

10.0 PLAN OF TREATMENT

10.1. Collection of autologous peripheral blood stem cells

We expect that all patients will be on some form of immune suppressive therapy at the time of their two visits to Seattle for eligibility screening. We do not want to deliberately cause a flare of symptoms and severity by discontinuing this therapy, but after consenting the patient for transplant, we will restage their Crohn's Disease while on therapy, then stop therapy before mobilization of autologous hematopoietic cells. This mobilization procedure employs G-CSF, prednisone and possibly cyclophosphamide (if there are problems with harvesting adequate numbers of cells); these medications are effective therapies in patients with Crohn's Disease. The rationale for collection of autologous hematopoietic cells is for storage and later use in the event of graft rejection after transplant.

10.1.1. Mobilization Schedule

The mobilization schedule is outlined in Table 1 below. Peripheral blood stem cells will be collected after treatment with G-CSF at a dose of 16 μg/kg/day. G-CSF dosing can be rounded based on subject's **actual body weight** and available G-CSF vial sizes to best approximate 16 μg/kg. CD34+ cell content in peripheral blood will be measured daily from Day 4 of G-CSF treatment (5 mL peripheral blood in heparin). Leukapheresis will be performed on a continuous flow cell separator according to institutional standards. Daily leukapheresis collection will continue until a minimum of 2.0 x 10^6 CD34+ cells/kg have been collected and cryopreserved.

Institutional standards for supportive care will be followed during mobilization and leukapheresis.

Table 1: Stem Cell Mobilization with G-CSF

Day	0	1	2	3	4	5
Prednisone (1 mg/kg/day) [a]	X	X	X	X	X	X
G-CSF (16 μg/kg/day)		X	X	X	X	X[b]
Peripheral Blood CD34 Assay					X	
Leukapheresis					X	X[b]

[a] For a total of 10 days.

[b] Day 5 G-CSF and leukapheresis required unless ≥ 2.0 x 10^6 CD34+ cells/kg cryopreserved after the first leukapheresis. It is expected that a maximum of 4 leukaphereses will be required to achieve target dose of ≥ 2.0 x 10^6 CD34+ cells/kg cryopreserved.

To prevent possible relapses or exacerbations of CD secondary to G-CSF, all subjects will be treated with prednisone 1 mg/kg/day, starting 1 day before the start of G-CSF. Prednisone will be continued for a total of 10 days. If there is evidence of an exacerbation or flare of CD during mobilization, the G-CSF will be stopped and the transplant will be delayed until the subject is reassessed for continued eligibility.

10.1.2. Second Mobilization with Cyclophosphamide if Failure to Mobilize with G-CSF and Prednisone

If after 4 collections there is a failure to reach the minimum requirement of 2 x 10^6 CD34+ cells/kg, leukapheresis collections may be stopped at the discretion of the attending physician. Two weeks later, a subsequent attempt at mobilization may be undertaken using cyclophosphamide and G-CSF (Table 2). For subjects requiring the repeat attempt at mobilization, cyclophosphamide 2.0 g/m^2 IV will be administered on Day 0. Seventy-two hours later, the subject will begin daily G-CSF (10 μg/kg/day subcutaneously). It is recommended that leukapheresis collection begin when the CD34+ cell content is 20,000/mL or as soon as feasible thereafter. Daily G-CSF will continue and leukapheresis will be performed daily as necessary to reach the minimum number of CD34 + cells (> 2.0 x 10^6 $CD34^+$/kg). If unsuccessful, other strategies for collection of stem cells will be reviewed with the PI.

Table 2: Stem Cell Mobilization with Cyclophosphamide and G-CSF

Day	0	1	2	3	4	5	10+	Subsequent Days
Cyclophosphamide 2g/m^2	X							
G-CSF (10 µg/kg/day)				X	X	X	X	X[b]
CBC and Differential							X	X[b]
Peripheral Blood CD34 Assay						X	X	
Leukapheresis[a]								X[b]

[a] It is recommended that leukapheresis continues until > 2.0 x 10^6 CD34 + cells/kg subject weight are cryopreserved (it is expected that a maximum of 4 leukaphereses will be required to collect sufficient CD34+ cells).

[b] If required.

10.1.3. Crohn Disease Exacerbation or Flare During Mobilization

If there is an exacerbation or flare of CD during mobilization, G-CSF will be stopped. The subject may be treated with high-dose corticosteroids or other disease-modifying therapy at the discretion of the treating physician. Prior to continuing on protocol, the patent will undergo repeat gastrointestinal assessment for status of CD. To continue on protocol, the subject will have to meet the original eligibility criteria.

10.2. Pre-conditioning medications

10.2.1. Ursodiol

Ursodiol (ursodeoxycholic acid) will be given orally at a dose of 12-15 mg/kg/day, in divided doses, starting 2 weeks before the start of conditioning therapy, and continued through day 140 after transplant. The purpose of prophylactic ursodiol is to prevent or ameliorate the effects of cholestatic liver disease [91,92].

10.2.2. N-acetyl cysteine

N-acetyl cysteine (NAC) replenishes hepatocyte mitochondrial and cytosolic glutathione stores, and because sinusoidal endothelial cell GSH is derived from hepatocytes, sinusoidal endothelial cell GSH is also replenished. Both oral and intravenous NAC are agents with few significant side effects found during clinical human trials in Europe and the USA. Intravenous NAC is approved for the treatment of acute liver failure caused by acetaminophen overdoses, for acute liver failure of other causes [100], and for a wide range of other medical conditions including chronic lung disease [101,102]. The rationale for its use before the start of conditioning therapy in this protocol is as follows: Patients with CD may come to transplantation in a malnourished state or following therapies that might affect hepatic GSH stores. Although the reduced-intensity conditioning regimen below is not notably toxic to sinusoidal endothelial cells, it may cause GSH depletion to some extent, and will be followed by cyclophosphamide in doses that are potentially toxic to sinusoidal endothelial cells [103]. Animal studies of a toxin that closely mimics cyclophosphamide's effect on sinusoidal endothelial cells show that GSH depletion worsens the toxicity and GSH repletion prevents it [90]. Thus, the administration of NAC at a dose of 600 mg BID (Twinlab, Hauppauge, New York) for two weeks before the start of conditioning therapy through day +5 post transplant is intended to replenish hepatocyte and sinusoidal endothelial cell GSH in order to prevent sinusoidal injury from the toxin cyclophosphamide.

10.3. Hematopoietic cell transplant schedule of medications and irradiation

Table 3. Schedule of medications and irradiation from day -6 through day +5.

Day	*Treatment*
-6	*Fludarabine 30 mg/M^2 + Cyclophosphamide 25 mg/kg + Mesna 25 mg/kg IV*
-5	*Fludarabine 30 mg/M^2 + Cyclophosphamide 25 mg/kg + Mesna 25 mg/kg IV*
-4	*Fludarabine 30 mg/M^2*
-3	*Fludarabine 30 mg/M^2*
-2	*Fludarabine 30 mg/M^2*
-1	*TBI 200 cGy*
0	*T cell replete marrow transplant*
+3	*Cyclophosphamide 50 mg/kg + Mesna 50 mg/kg IV*
+4	*Cyclophosphamide 50 mg/kg + Mesna 50 mg/kg IV*
+5	*Tacrolimus + Myfortic or Mycophenolate mofetil + G-CSF*

10.3.1. Fludarabine

Fludarabine 30 mg/m^2/day will be administered over 30-60 minutes intravenous infusion on Days –6 through –2. Fludarabine will be dosed according to the recipient's actual body weight, unless the actual body weight is greater than or equal to two times their ideal body weight, in which case the Protocol Team must be consulted for instruction.

For decreased creatinine clearance (< 61 mL/min) determined by the Cockcroft Formula:

$$C_{Cr} = \frac{(140 - \text{age}) \times \text{ideal body weight (IBW) (kg)} \times 0.85 \text{ (for women)}}{\text{PCr} \times 72}$$

Fludarabine dosage should be reduced as follows:

C_{Cr} 46-60 mL/min, fludarabine = 24 mg/m2
C_{Cr} 31-45 mL/min, fludarabine = 22.5 mg/m2
C_{Cr} 21-30 mL/min, fludarabine = 19.5 mg/m2
C_{Cr} < 20 mL/min, fludarabine = 15 mg/m2

10.3.2. Pre-transplant Cyclophosphamide

CY 25 mg/kg/day will be administered as a 1-2 hour intravenous infusion with a high volume fluid flush on Days –6 and –5. CY will be dosed according to the recipient's adjusted body weight (see 10.3.5 for formulas). Please follow FHCRC Standard Practice Guidelines for weight adjustment/infusion guidelines of cyclophosphamide.

Mesna will be given in divided doses IV 30 min pre- and at 3, 6, and 8 hours after each dose of cyclophosphamide. Mesna dose is based on the cyclophosphamide dose being given. The total daily dose of mesna is equal to 100% of the total daily dose of cyclophosphamide.

10.3.3. Total Body Irradiation

Total body irradiation: 200 cGy will be administered in a single fraction on Day –1 from a linear accelerator at a rate of 6-15 cGy/min.

10.3.4. Marrow Transplantation

Patients will receive T cell replete marrow on day 0. If there is a major ABO incompatibility, the red blood cells will be depleted from the donor marrow. Donor bone marrow will be harvested with a target yield of 4×10^8 nucleated cells/kg recipient IBW. A sample of the product to be infused will be sent for flow cytometry to determine the content of CD34+ CD3+, CD4+, and CD8+ cells.

10.3.5. Post-transplant Cyclophosphamide

Hydration for high-dose cyclophosphamide administration may be given according to institutional standards.

Mesna will be given in divided doses IV 30 min pre- and at 3, 6, and 8 hours after each dose of cyclophosphamide. Mesna dose is based on the cyclophosphamide dose being given. The total daily dose of mesna is equal to 100% of the total daily dose of cyclophosphamide.

Cyclophosphamide [50 mg/kg IBW] will be given on Day 3 post-transplant (between 60 and 72 hours after marrow infusion) and on Day 4 post-transplant (approximately 24 hours after Day 3 cyclophosphamide). Cyclophosphamide will be dosed according to the recipient's adjusted body weight, (see below for formulas). Cyclophosphamide will be given as an IV infusion over 1-2 hours. Please follow FHCRC Standard Practice Guidelines for weight adjustment/infusion guidelines of cyclophosphamide.

Ideal Body Weight (IBW) Formulas:

Male Ideal Body Weight = 50 kg + 2.3 kg/inch over 5 feet
Female Ideal Body Weight = 45.5 kg + 2.3 kg/inch over 5 feet

Adjusted Body Weight Formula:

Adjusted Body Weight = Ideal Body Weight + [(0.25) x (Actual Body Weight – Ideal Body Weight)]

No immunosuppressive agents should be given until 24 hours after the completion of the post-transplant cyclophosphamide. This includes corticosteroids such as dexamethasone as anti-emetics.

10.3.6. Tacrolimus

Tacrolimus will be given at a dose of 1 mg IV daily or 1 mg po twice daily from day +5 until day +180. If tacrolimus is initiated using the IV formulation it will be changed to a PO dosing schedule once a therapeutic level is achieved. Serum levels of tacrolimus will be measured around day +7 and then should be checked at least weekly thereafter and the dose adjusted accordingly to maintain a level of 5-15 ng/mL to approximately day +180. Tacrolimus will be tapered starting at approximately day +180 and then discontinued on approximately day +365. Tacrolimus may be continued if active GVHD is present. If there is nausea and vomiting at anytime during tacrolimus treatment, the drug should be administered intravenously and dose adjusted to the levels described above. Cyclosporine (target concentration 200-400 ng/mL) may be substituted for tacrolimus if the patient is intolerant of tacrolimus.

In the absence of GVHD or treatment for GVHD, tacrolimus is to be tapered 25% on approximately day +180 and then 25% every 2 months until discontinued at day 365. The referring physician, who will receive explicit instructions and guidelines for detecting and managing GVHD, may manage this tacrolimus taper.

Blood pressure, renal function (assessed with serum creatinine, BUN), electrolytes and magnesium should be followed three times per week during the first month, twice weekly until day +100, then once per week until tacrolimus is tapered off (or according to institutional practice), unless clinical circumstances suggest the need for more frequent evaluations.

Monitoring and Dose Adjustments - Whole blood "trough" levels (i.e. 11-12 hours from the prior dose) of tacrolimus will be evaluated weekly until day +180. Dose reductions should be made if tacrolimus toxicity (such as renal insufficiency) is present, or levels exceed 15 ng/ml in the absence of toxicity on more than 2 occasions. If toxicity or high levels are present, more frequent monitoring will be initiated as clinically indicated. Dose reductions for high levels with or without toxicity should be conservative, e.g. 25%, to avoid inadequate immune suppression.

Drugs that may affect tacrolimus levels: dilantin, phenobarbital, rifampicin (may lower tacrolimus levels); and glucocorticoids, fluconazole, ketoconazole, itraconazole, voriconazole, cimetidine (may increase tacrolimus levels). Discontinuation of fluconazole will likely lower tacrolimus levels.

10.3.7. Mycophenolic acid enteric coated (Myfortic) or Mycophenolate Mofetil

The enteric formulation of mycophenolate sodium (Myfortic) that delivers the active moiety of mycophenolic acid is associated with less gastrointestinal side effects than MMF. Myfortic will be dosed by actual body weight at a dose of 11 mg/kg PO TID. The drug is supplied as 180 mg and 360 mg tablets. The maximum total daily dose should not exceed 2,160 mg. If there is nausea and vomiting at any time preventing the oral administration of Myfortic, MMF should be administered intravenously. If an IV formulation is required, MMF will be used at a dose of 15 mg/kg. The maximum daily dose should not exceed 3,000 mg. Myfortic or MMF will be discontinued after the last dose on Day 35, or may be continued if active GVHD is present. Myfortic doses will be rounded to the nearest 180 mg.

Guidelines for Myfortic or MMF dose adjustment: If in the clinical judgment of the investigator the observed toxicity is related to Myfortic or MMF administration, a dose adjustment will occur. Based on previous organ transplant studies, dose adjustments are likely to occur because of hematopoietic or gastrointestinal adverse effects. Dose adjustments will not be made for hematopoietic toxicity unless severe neutropenia develops or persists after day 21 post transplant (ANC < 7500/μL). In the event of gastrointestinal toxicity that requires medical intervention including medication for control of persistent vomiting or diarrhea that is considered to be due to Myfortic, a 20% dose reduction will occur or MMF may be given IV. For severe G.I. toxicity related to Myfortic (severe refractory diarrhea, or overt gastrointestinal bleeding), the Myfortic or MMF may be temporarily stopped.

10.4. Growth Factor Support

G-CSF will be given beginning on Day 5 at a dose of 5 mcg/kg/day (rounding to the nearest vial dose is allowed), until the absolute neutrophil count (ANC) is >1,000/mm3 for three consecutive days.

10.5. Transfusion Support

Platelet and packed red cell transfusions will be given per FHCRC standard of practice. Platelet counts will be maintained >20,000 after transplant to prevent bleeding from the gastrointestinal tract.

10.6. Anti-Ovulatory Treatment

Menstruating females should be started on an anti-ovulatory agent before the initiation of the conditioning regimen, as outlined in the FHCRC Standard Practice Manual.

10.7. Nutritional Support

Patients will receive nutritional support according to FHCRC Standard Practice Guidelines including total parenteral nutrition through a central venous catheter if unable to maintain body weight by oral caloric intake.

10.8. Infection Prophylaxis Guidelines

All prophylactic antibiotics may be changed or discontinued according to clinical circumstances (such as subject allergy) as determined by attending physician(s). If there are discrepancies between the recommendations below (reflecting current recommendations) and revised Standard Practice Guidelines, the extant FHCRC Standard Practice Guidelines for infection prophylaxis will be followed in this protocol,

10.8.1. Antibacterial Prophylaxis

If not already on broad-spectrum antibiotics, once neutropenia (neutrophils < $0.5x10^9$/L) occurs, prophylactic broad-spectrum oral antibiotics will be started. Levofloxacin is the standard antibiotic for prophylactic use in adults over 18 years of age.

10.8.2. Antifungal Prophylaxis

In general, the guidelines in the Standard Practice Manual will be followed, to wit: Fluconazole 400 mg PO will be administered daily from start of conditioning therapy until day +75 post-transplant. Begin vorlconazole if there is a pretransplant history of invasive aspergillosis or evidence of colonization with Aspergillus species or a Galactomannen test suggesting aspergillus infection. Post-transplant, if patients develop GVHD and require treatment with prednisone or methylprednisolone ≥1 mg/kg, stop fluconazole and start posaconazole 200 mg TID PO. If patient is unable to take oral agents, then start voriconazole 4 mg/kg intravenously BID.

10.8.3. Antiviral Prophylaxis

For patients who weigh ≥40 kg, valacyclovir (500 mg PO BID) or equivalent will be administered from the start of conditioning therapy to day 365 post-transplant for patients who are serologically positive for VZV. For patients

weighing <40 kg, the dose of valacyclovir will be 250 mg PO BID. Antiviral therapy will be given to day +100 for patients who are serologically negative for VZV but serologically positive for HSV. Acyclovir 250 mg/m^2 q 12 hr IV will be substituted in subjects unable to tolerate oral medication. Acyclovir/valacyclovir doses will be adjusted according to renal function.

10.8.4. Anti-Pneumocystis Prophylaxis

For adults, trimethoprim-sulfamethoxazole DS tablet will be given PO BID 2 days per week or equivalent, starting after engraftment and continuing for 1 year after transplant. Subjects intolerant or allergic to trimethoprim-sulfamethoxazole will be treated with dapsone, pentamidine, or atovaquone according to local institutional standard of practice. Patients will be treated beyond 1 year if chronic GVHD develops requiring treatment.

10.8.5. Cytomegalovirus

Subjects who are CMV **seronegative** will be administered CMV-negative screened blood products or leuko-depleted blood products. The subjects will be monitored with a pretransplant test and then monitored weekly for CMV reactivation using CMV antigenemia or CMV DNA assays until day +60 after transplant. For patients who have CMV diagnosed before day +60, patients will continue to be monitored until 1 year after transplant.

Subjects who are CMV **seropositive or who have seropositive donors** will be monitored with weekly for CMV reactivation using CMV antigenemia or CMV DNA assays post-HCT beginning on about day 0 and continuing until discharge or day +100. CMV monitoring will occur at least every other week during follow-up visits until **6 months** post-HCT. For those patients that have reactivated CMV before day 100, or for those who are taking prednisone for chronic GVHD after day 100, weekly CMV monitoring will continue to 1 year after transplant according to institutional practice.

Subjects treated for CMV reactivation should have weekly CMV antigenemia titers for 3 weeks prior to reinitiating every other week monitoring.

10.8.6. Epstein Barr Virus (EBV)

Baseline studies (pre-transplant):

1. Serology for antibodies, including antibody titers, to EBV viral capsid antigen and/or nuclear antigen
2. EBV PCR amplification to evaluate viral load in the peripheral blood, for individual subjects, when baseline EBV serology is negative

Post-transplant surveillance monitoring beginning at Day +14 post-transplant, through Day +100 post-transplant:

1. EBV PCR, perform weekly
2. EBV PCR, perform twice per week, if there is evidence of rising copy number

Post-transplant surveillance monitoring from Day 101 to 6 months post-transplant:

1. EBV PCR, perform every other week
2. If the test is positive, then perform EBV PCR twice per week.

Subjects who develop a viral load of > 1000 copies per mL plasma will receive further individualized monitoring and/or therapy.

10.9. Immunizations

At 1-year post-HCT, subjects should be initiated on a program of re-vaccination according to the FHCRC Standard Practice Manual.

Treatment of infectious diseases will be conducted according to the FHCRC standard of practice and the clinical judgment of the treating physician(s).

10.10. Treatment of Graft-vs-Host Disease

First-line therapy will be 1 or 2 mg/kg of prednisone or methylprednisolone. For patients who do not respond to treatment, second-line therapy will include additional immunosuppressive therapy as clinically indicated. Management of chronic GVHD will be based on the recommendations of physicians experienced in the care of transplant survivors with chronic GVHD. .

10.11. Management of Graft Rejection

It is expected that patients will have recovery of autologous hematopoiesis after graft rejection but may have a prolonged period of neutropenia. Patients will be monitored and treated appropriately to prevent opportunistic infections during this recovery period. Patients who experience protracted neutropenia caused by graft rejection will be reviewed by the FHCRC Patient Care Conference faculty where a decision will be made about a second transplant using a patient's autologous hematopoietic cells that had been stored before the start of conditioning therapy.

11.0 EVALUATION

11.1. First Phase Screening Visit

A consent document must be signed by the patient or an authorized parent or guardian before examinations and tests are begun, following counseling by a study investigator. The schedule of First Phase Screening Visit evaluations, as outlined below, can be found in Appendix G. The laboratory studies listed in the paragraphs 3., 4. and 5. below may be performed at the First Phase Screening visit at the discretion of the study investigators. Following this first visit, the Eligibility Review Group Documentation form (Appendix K) will be completed and approved by the Eligibility Review Group before scheduling the Second Phase Screening Visit.

1. Medical history and physical examination including vital signs, current medications, and detailed CD-focused documentation.
2. If no history of smoking or of cardiac or pulmonary problems or findings on physical examination, defer pulmonary and cardiac tests until the Second Phase Screening Visit. If there is a clinical suspicion of cardiopulmonary issues, pulmonary evaluations will include pulmonary function tests (including FVC, FEV1 and hemoglobin adjusted DL_{CO}) pre and post bronchodilator (if required) and resting O_2 saturation by pulse oximetry. Cardiac evaluations will include echocardiogram and electrocardiogram (ECG).
3. CBC with differential, comprehensive metabolic panel including uric acid, PT, PTT, direct Coomb's, ESR.
4. Renal evaluations include complete urinalysis (UA) and spot urine collection for evaluation of protein, creatinine, and albumin:creatinine ratio (ACR). Creatinine clearance will be calculated using serum creatinine and the Cockcroft Gault Formula.
5. Serum Quantitative-HCG pregnancy test for female subjects.
6. Completion of the Hematopoietic Cell Transplantation-Comorbidity Index (Appendix F).
7. Completion of the Crohn's Disease Activity Index and Harvey-Bradshaw Index (Appendix A).
8. Determination of Body Mass Index (http://www.nhlbisupport.com/bmi/bmi-m.htm)
9. If subjects have not had endoscopic biopsy confirmation of CD-related intestinal inflammation in the last 6 months before arrival in Seattle, they may undergo endoscopic evaluation and biopsy of mucosa in Seattle, or defer these procedures until they return to the care of their gastroenterologist or surgeon. In the event that there is no active inflammation within reach of standard esophagogastroduodenoscopy and colonoscopy/ileoscopy, determination of active inflammation will be made by CT enterography or another imaging method. Included in biopsy evaluation will be centrifugation culture for CMV and Adenovirus. Biopsy specimens in excess of those needed to determine the presence of active inflammation will be placed in a tissue bank for research purposes, as described in Section 11.2, #14.
10. Fertility counseling. Cryopreservation of sperm should be offered to fertile male subjects. Testing and storage of sperm should be undertaken prior to any treatment on study. Fertile female subjects should be specifically counseled as to risks of infertility and possibility of in vitro fertilization and storage of fertilized embryos.
11. To follow: HLA typing of patient and family members, upon review of eligibility criteria and insurance company approval of the procedure.

11.2. Second Phase Screening Visit and additional baseline testing

Patients who come to Seattle for this visit will have undergone favorable reviews by the Eligibility Review Group and their insurance companies and an eligible HLA-matched allogeneic donor will have been identified. Patients will be assigned to an allogeneic transplant team at the Seattle Cancer Care Alliance, and a consultation with the Gastroenterology/Hepatology Service pre-arranged. Unless more than 90 days have passed since the completion of the First Phase Screening Visit, or the patient has developed signs and symptoms that are changed from the first visit, some of the results of the first visit tests will be considered as baseline study assessments for subjects proceeding to transplant, to which will be added information from the Second Phase Screening Visit.

1. Interval medical history and physical examination including vital signs and current medications.
2. Dental evaluation (with referral to oral surgeon for abscess or infection if indicated).
3. Evaluation for CD-related quality of life (SIBDQ , Appendix B)
4. Pulmonary function tests (including FVC, FEV1 and hemoglobin adjusted DL_{CO}) pre and post bronchodilator (if required) and resting O_2 saturation by pulse oximetry.
5. Cardiac evaluations will include echocardiogram and electrocardiogram (ECG), if not done at the first screening visit.
6. Magnetic resonance imaging, CT enterography or a comparable intestinal imaging method
7. Endoscopic evaluation and biopsy of mucosa, unless biopsies demonstrating CD-related inflammation had been demonstrated at the First Phase Screening visit within the previous 6 months. In the event that there is no active inflammation within reach of standard esophagogastroduodenoscopy and colonoscopy/ileoscopy, determination of active inflammation will be made by CT enterography or another imaging method. Included in biopsy evaluation will be centrifugation culture for CMV and Adenovirus. Biopsy specimens in excess of those needed to determine the presence of active inflammation will be placed in a tissue bank (see section 11.1, #9).
8. Stool specimen for toxigenic Clostridium difficile by PCR, ova and parasite examination (including cryptosporidia and microsporidia), Giardia antigen, and enteric viruses. Stool specimens will also be collected for future studies of the intestinal microbiome, as follows: Subjects will be provided with stool hats for obtaining fecal samples. Fecal samples will be processed using a straw core technique. Straw cores will be pushed using a wooden stick into 2 ml screw cap cryovials containing 1 cc of the following solutions and frozen at -80 degrees C. These solutions will include PowerBead Solution with 0.1 glass beads; PBS:40% glycerol; and RNAlater. Additional stool cores will be placed in 4 cryovials without any solutions added.
9. CD Activity Index and Harvey-Bradshaw Index (Appendix A)
10. Clinical blood draw for CBC with differential, comprehensive metabolic panel, INR, quantitative immunoglobulins, anti-hepatitis C virus, hepatitis B surface antigen, anti-hepatitis B core, HIV, T4, TSH, antibodies to CMV, HSV, and VZV.
11. Renal evaluations include complete urinalysis (UA) and spot urine collection for evaluation of protein, creatinine, and protein:creatinine ratio. Creatinine clearance will be calculated using serum creatinine and the Cockcroft-Gault formula.
12. Serum pregnancy test (Quantitative-HCG).
13. Research blood draws: Approximately 100 mL of blood will be drawn and archived for later research, briefly described below. Blood will be drawn into a 10 mL plain tube for serum, a 10 mL heparinized tube for plasma plus DNA, a 3 ml Tempus tube for mRNA, a 10 mL potassium oxalate-containing tube for plasma, and six 10 ml heparinized tubes for peripheral blood mononuclear cells (PBMC). Tempus tubes, serum and plasma will be frozen at -80°C. Serum tubes will be centrifuged, and plasma will be extracted and likewise frozen, while buffy coat cells will be harvested and lysed so their DNA may be extracted and frozen. The remaining tubes will be centrifuged over Ficoll to isolate PBMC, which will be frozen gradually in 7% DMSO and stored in liquid nitrogen. DNA will be analyzed via immunochip for SNP's associated with CD and other autoimmune diseases. Serum will be analyzed for serological markers of CD, including C reactive protein and antibodies to intestinal microflora antigens. Plasma will also be analyzed by Luminex microbead assay for content of a panel of cytokines made by Th1, Th2, Th17, and Treg cells, as well as by cells of the innate immune system. PBMC will be analyzed by flow cytometry, using 12-color antibody panels that identify T cell and innate immune cell subsets, and will also be stimulated in vitro to analyze cytokine production by intracellular staining.

14. Research endoscopic biopsies: Four biopsy specimens will be taken from stomach, duodenum, ileum and/or colon, depending on the endoscopic procedure. If both inflamed and uninflamed mucosa of one or more of the above anatomic sites are noted grossly, then 4 biopsies each from inflamed and uninflamed tissue) will be obtained. For each set of 4 biopsies, one will be snap-frozen for mRNA analysis. One will be frozen embedded in OCT for immunohistology. Then each of the remaining 2 biopsies will be gradually frozen in 7% DMSO/FCS to allow release and analysis of viable lymphocytes at a future date.

After the second visit has verified that eligibility criteria have been met, and the Eligibility Review Group has confirmed that the subject has met eligibility criteria (see Appendix E) subjects will then undergo final **Pre-transplant tests, counseling and informed consent** (Section 11.3)

11.3. Pre-transplant tests, counseling and informed consent

If a review of results of the first and second screening visits confirms eligibility, subjects will proceed to the final steps toward transplantation. However, certain testing may need to be repeated at the discretion of the investigators as clinically indicated to ensure patient safety and to re-establish baseline values.

1. Interval history and physical examination, including vital signs.
2. Safety Laboratory Tests: CBC with differential, comprehensive metabolic panel, and serum Quantitative-HCG pregnancy test.
3. CMV DNA (by PCR)
4. Screening should be done for respiratory viruses (RSV, parainfluenza, and influenza) with nasal wash and throat swab prior to initiating the conditioning regimen. If the screen is positive, transplant should be delayed for 3-4 weeks until repeat cultures/PCR are negative.
5. Counseling about the transplant procedure by a transplant attending physician (Allogeneic Transplant Service).
6. Signing of an informed consent document for allogeneic hematopoietic cell transplantation.
7. Placement of a two-lumen Hickman catheter.

11.4. Study Procedures from Time of Initiation of Hematopoietic Cell Transplant

While in Seattle and until discharge to the care of each patient's provider(s), all hospitalized patients are evaluated daily by transplant physicians at the University of Washington Medical Center or Seattle Children's Hospital, and all out-patients are evaluated frequently (at least once weekly, depending on medical issues) by transplant physicians at the Seattle Cancer Care Alliance. All patients are expected to be housed within a 30 minute journey to the Seattle Cancer Care Alliance outpatient facility. The frequency of standard laboratory tests and radiologic studies following transplantation will be those in the FHCRC Standard Practice Manual. Additional evaluations, including clinical, laboratory, endoscopic, and imaging tests, will be undertaken for exigent clinical circumstances. The following protocol-specific assessments will be undertaken at months 1, 3, and 12 after transplant and at yearly intervals thereafter for 5 years.

11.4.1 Clinical assessments at Month 1 (Day 28 ± 4 days)

The following evaluations will be performed at Month 1 (Day 28; +/- 4 days) after allogeneic HCT.

1. Detailed physical examination.
2. CBC with differential and comprehensive metabolic panel.
5. CMV DNA.

11.4.2 Clinical assessments at Month 3 (Day 80 ± 5 days)

1. Detailed physical examination.
2. PFTs with DLCO and FVC and resting pulse oximetry for O_2 saturation.
3. Endoscopic evaluation and biopsy of intestinal mucosa. In the event that there is no active inflammation within reach of standard esophagogastroduodenoscopy and colonoscopy/ileoscopy, determination of active inflammation will be made by magnetic resonance imaging, CT enterography or another imaging method. Included in biopsy evaluation will be centrifugation culture for CMV and Adenovirus. Biopsy

specimens in excess of those needed to determine the presence of active inflammation will be placed in a tissue bank (see section 11.1, #9).

4. CD activity index and Harvey-Bradshaw Index
5. SIBDQ QOL instrument
6. CBC with differential and comprehensive metabolic panel.
7. Renal evaluations include complete urinalysis (UA) and spot urine collection for evaluation of protein, creatinine, and albumin:creatinine ratio. Creatinine clearance will be calculated using serum creatinine and the Cockcroft-Gault formula.
8. Body Mass Index
9. Quantitative Immunoglobulins (Serum IgG, IgM, and IgA).
10. CMV DNA as per FHCRC Standard Practice Manual and EBV DNA per protocol.
11. Chimerism studies of CD33+ and CD3+ cells in peripheral blood.
12. Vaccine antibody titers per FHCRC Standard Practice Manual.
13. Evaluation for chronic Graft-vs-Host Disease, per FHCRC Standard Practice Manual (Appendix H).
14. Research blood draws: see section 11.2, #13.
15. Research stool collection: Stool specimens will also be collected for future studies of the intestinal microbiome, as follows: Subjects will be provided with stool hats for obtaining fecal samples. Fecal samples will be processed using a straw core technique. Straw cores will be pushed using a wooden stick into 2 ml screw cap cryovials containing 1 cc of the following solutions and frozen at -80 degrees C. These solutions will include PowerBead Solution with 0.1 glass beads; PBS:40% glycerol; and RNAlater. Additional stool cores will be placed in 4 cryovials without any solutions added.

11.4.3. Clinical assessments at Month 12 (Day 365 ± 20 days)

1. Detailed physical examination.
2. CT enterography or a comparable intestinal imaging method
3. Endoscopic evaluation and biopsy of intestinal mucosa. In the event that there is no active inflammation within reach of standard esophagogastroduodenoscopy and colonoscopy/ileoscopy, determination of active inflammation will be made by magnetic resonance imaging, CT enterography or another imaging method. Included in biopsy evaluation will be centrifugation culture for CMV and Adenovirus. Biopsy specimens in excess of those needed to determine the presence of active inflammation will be placed in a tissue bank (see section 11.1, #9).
4. CD activity index and Harvey-Bradshaw Index
5. SIBDQ QOL instrument
6. CBC with differential and comprehensive metabolic panel.
7. Renal evaluations include complete urinalysis (UA) and spot urine collection for evaluation of protein, creatinine, and albumin:creatinine ratio. Creatinine clearance will be calculated using serum creatinine and the Cockcroft-Gault formula.
8. Pulmonary function tests
9. Body Mass Index
10. Quantitative Immunoglobulins (Serum IgG, IgM, and IgA).
11. Chimerism studies of CD33+ and CD3+ cells in peripheral blood.
12. Evaluation for chronic Graft-vs-Host Disease, per FHCRC Standard Practice Manual (Appendix G).
13. Vaccine antibody titers per FHCRC Standard Practice Manual.
14. Research blood draws: see section 11.2, #13.

15. Research stool collection: Stool specimens will also be collected for future studies of the intestinal microbiome, as follows: Subjects will be provided with stool hats for obtaining fecal samples. Fecal samples will be processed using a straw core technique. Straw cores will be pushed using a wooden stick into 2 ml screw cap cryovials containing 1 cc of the following solutions and frozen at -80 degrees C. These solutions will include PowerBead Solution with 0.1 glass beads; PBS:40% glycerol; and RNAlater. Additional stool cores will be placed in 4 cryovials without any solutions added.

11.4.4. Clinical assessments at Years 2 through 5.

Although highly desirable, it may not be practical for subjects to return to Seattle annually after the first year. In this case, the CDAI and SIBDQ will be. administered by study investigators by telephone, mail, or email correspondence, and blood specimens for chimerism studies and research blood draws will be sent to FHCRC for processing. The detailed physical examination and endoscopic or imaging evaluations at years 3 and 5 will be done in Seattle if possible, but if travel to Seattle is not feasible, these procedures can be carried out locally, following protocol guidelines, and the results reviewed by study investigators. Tissue specimens will be sent to the FHCRC for independent evaluation.

1. Detailed physical examination.
2. At years 3 and 5, or at any time that there are clinical signs and symptoms that could indicate recurrent CD, endoscopic evaluation and biopsy of intestinal mucosa. In the event that there is no active inflammation within reach of standard esophagogastroduodenoscopy and colonoscopy/ileoscopy, determination of active inflammation will be made by magnetic resonance imaging, CT enterography or another imaging method. Included in biopsy evaluation will be centrifugation culture for CMV and Adenovirus. Biopsy specimens in excess of those needed to determine the presence of active inflammation will be placed in a tissue bank (see section 11.1, #9).
3. CD Activity Index and Harvey-Bradshaw Index
4. SIBDQ
5. CBC with differential and comprehensive metabolic panel.
6. Renal evaluations include complete urinalysis (UA) and spot urine collection for evaluation of protein, creatinine, and albumin:creatinine ratio. Creatinine clearance will be calculated using serum creatinine and the Cockcroft-Gault formula.
7. Quantitative Immunoglobulins (Serum IgG, IgM, and IgA).
8. Chimerism studies of CD33+ and CD3+ cells in peripheral blood (to be carried out at FHCRC).
9. Research blood draws: see section 11.2, #13.
10. Research stool collection: Stool specimens will also be collected for future studies of the intestinal microbiome, as follows: Subjects will be provided with stool hats for obtaining fecal samples. Fecal samples will be processed using a straw core technique. Straw cores will be pushed using a wooden stick into 2 ml screw cap cryovials containing 1 cc of the following solutions and frozen at -80 degrees C. These solutions will include PowerBead Solution with 0.1 glass beads; PBS:40% glycerol; and RNAlater. Additional stool cores will be placed in 4 cryovials without any solutions added.

11.4.5. Evaluation at an early withdrawal visit

If a patient withdraws from the study prematurely, an Endpoint Evaluation Visit will be carried out as described for the Year 1 evaluation (see 11.4.3). Our Long-Term Follow-Up section follows all of our past transplant patients for life, through yearly questionnaires (per our Standard Practice Manual) and through communication with physicians who care for our former patients--unless patients specifically designate their desire to not participate in any way in follow-up.

12.0 DRUGS, IRRADIATION AND MARROW/STEM CELL ADMINISTRATION TOXICITIES AND COMPLICATIONS

12.1. G-CSF for autologous PBSC collection

Granulocyte-Colony Stimulating Factor (G-CSF) is a growth factor given to mobilize hematopoietic stem cells into

the peripheral blood and boost granulocyte counts. It has been used for the collection of stem cells in normal allogeneic donors as well as in candidates for autologous transplants. It has been administered to subjects with primary or acquired granulocyte deficiencies. Most frequently it is used to aid granulocyte recovery after drug-induced myelosuppression, including conventional cytotoxic chemotherapy for cancer and also after marrow transplantation. G-CSF is produced by recombinant DNA technology and acts by binding to specific receptors on hematopoietic cells. It stimulates proliferation, differentiation, and some functional activities of granulocytes and their precursors. G-CSF is administered either by subcutaneous injection or intravenous infusion. Elimination half-life is approximately 3.5 hours. The primary toxicities of G-CSF are usually mild and include symptoms of myalgia and bone pain. Rarely splenic rupture has been reported. Vasculitis has been reported, and inflammatory processes or autoimmune diseases may be exacerbated. G-CSF has been studied as a treatment for CD, as immune cells exposed to G-CSF can develop a Th-2 response-that decreases the inflammatory Th-1 response in CD.

12.2. Cyclophosphamide

Cyclophosphamide is an alkylating agent used to treat a variety of malignancies. Cyclophosphamide (total dose 50 mg/kg) will be given as part of the conditioning regimen before infusion of donor bone marrow to reduce the risk of graft rejection and given again early after bone marrow transplant at a total dose of 100 mg/kg to prevent GVHD. It requires hepatic metabolism to the active metabolite phosphoramide mustard which reacts with nucleophilic groups and leads to immune suppression.. The half-life of the parent compound is 5.3 hours in adults, and the half-life of the major metabolite, phosphoramide mustard, is 8.5 hours. Liver or renal dysfunction will lead to prolonged serum half-life. When given in high doses after allogeneic HCT, cyclophosphamide targets proliferating alloreactive T cells and successfully prevents GVHD in mouse models and humans [104]. It has been hypothesized that the timing of the high-dose, post-transplant cyclophosphamide results in selective killing of activated alloreactive T cells while sparing resting T cells specific for infectious agents. The major dose-limiting side effect at high doses is cardiac necrosis but this is an unusual complication in patients with normal cardiac function pre-transplant. Hemorrhagic cystitis can occur and is mediated by the acrolein metabolite; it can be prevented by co-administration of Mesna or by continuous bladder irrigation. Other side effects include nausea, vomiting, alopecia, sinusoidal liver damage, myelosuppression, infertility, secondary malignancies, and syndrome of inappropriate antidiuretic hormone secretion (SIADH).

12.3. Fludarabine

Fludarabine is an antimetabolite with significant immunosuppressive activity. A total dose of 150 mg/m^2 will be given in the conditioning regimen. It is a highly immunosuppressive agent and is being given to prevent graft rejection. Fludarabine monophosphate is a purine nucleoside analogue that, after administration, undergoes rapid conversion in plasma to 2-fluoro ara-A (F-araA). F-araA subsequently enters cells where it is phosphorylated to F-araATP and the monophosphate F-araAMP. Once activated, F-araATP inhibits DNA polymerase and ribonucleotide reductase. The monophosphate F-araAMP, once incorporated into DNA, is an effective DNA chain terminator. Following IV administration, the drug is metabolized to 2-F-araA and widely distributed in tissues. 1-F-araA is excreted primarily in urine and has a terminal elimination half-life of 7-12 hr. The dose of fludarabine used in this protocol is myelosuppressive but not myeloablative. It also causes significant, prolonged immunosuppression by in vivo depletion of especially CD4+ cells. The immunosuppression associated with the use of fludarabine increases the risk of infection which can be life threatening. Other side effects associated with fludarabine include anorexia, nausea, vomiting, diarrhea and stomatitis. Neurological side effects observed at high doses include agitation, visual disturbances, confusion, coma, peripheral neuropathies. With high doses, confusion, blindness, coma and death have been reported (when used in doses of at least 120 mg/m2 daily for 4-5 days or a total dose of ≈ 500 mg/m2).

12.4. Total Body Irradiation (TBI)

TBI will be given in one 2.0 Gy fraction from a linear accelerator at a rate of 6-15 cGy/min. Although this is a low-dose of TBI, studies in dogs and humans have shown that it contributes significantly to the immunosuppression required to prevent graft rejection after allogeneic hematopoietic cell transplantation. Dosimetry calculations are performed by the radiation therapist. TBI may be associated with nausea, vomiting, and diarrhea; decline in hematocrit, platelet and white blood cell counts, and infection. Pulmonary, brain, hepatic toxicity and second malignancies have also been associated with TBI at higher doses. The risks and severity of many these effects are dose-related.

12.5. Tacrolimus

Tacrolimus is an immunosuppressive agent used to prevent and treat GVHD as well as prevent graft rejection after allogeneic HCT in hematopoietic cell transplant recipients. It is also used prevent the rejection of

transplanted kidneys, hearts and livers. The drug inhibits T-lymphocyte function with minimal activity against B-cells. Tacrolimus will be administered after posttransplant high-dose cyclophosphamide in combination withMyfortic or MMF. A calcineurin inhibitor in combination with MMF has been used routinely to prevent GVHD and rejection after reduced-intensity conditioning regimens and allogeneic HCT. Nephrotoxicity is the most frequent side effect of tacrolimus. Other frequently observed side effects include hypertension, dizziness, headache, insomnia, tremor, pruritus, rash, diabetes mellitus, hyperkalemia, hyperlipemia, hypomagnesemia, hypophosphatemia, renal tubular acidosis, constipation, diarrhea, dyspepsia, nausea, vomiting, arthralgia, back pain, weakness and paresthesia. Patients are also at risk of neurological complications including agitation, amnesia, anxiety, confusion, depression, encephalopathy, hallucinations, somnolence and psychosis. A characteristic complication associated with calcineurin inhibitors is posterior reversible encephalopathy syndrome (PRES). Patients with PRES present with headache, visual disturbance, hypertension and characteristic changes on brain MRI.

12.6. Mycophenolic acid enteric coated (Myfortic) or Mycophenolate Mofetil

Mycophenolic acid is an immunosuppressive agent, used in this protocol to prevent allograft rejection and GVHD. It can be delivered as an enteric formulation of mycophenolate sodium or MMF Previous clinical studies in patients after renal allografting suggested that the principal adverse reactions associated with the administration of MMF include diarrhea, leukopenia, sepsis, vomiting and possibly a higher incidence of certain viral infections (CMV, VZV, Herpes Simplex). In the setting of marrow transplantation, MMF has an increased incidence of digestive system adverse events, including GI tract ulceration, and hemorrhage (3% of patients receiving MMF). GI tract perforations have rarely been observed. Most patients in these studies were also on other drugs known to be associated with these complications. Myfortic has been shown to reduce the risk of gastrointestinal complications. Up to 2% of patients receiving MMF for prevention of rejection developed severe neutropenia (ANC <50/μL). The development of neutropenia may be related to MMF itself, concomitant medications, viral infections or some combination of these causes.

12.7. Allogeneic Marrow Transplantation (GVHD and Graft rejection)

We have hypothesized that allogeneic HCT will result in a sustained remission of CD by either 1) the elimination of the autoreactive host immune effector cells if full donor hematopoietic chimerism is established, or 2) modulation of the regulatory immune activity if mixed hematopoietic chimerism is established . Sustained remissions of autoimmune diseases have been observed in recipients with mixed hematopoietic chimerism after allogeneic HCT.

We will use marrow rather than peripheral blood stem cells as the source of the allogeneic hematopoietic cell graft to reduce the risk of chronic GVHD.
The major transplant-related complications are GVHD, graft rejection and delayed immune reconstitution which increases the risk of opportunistic infections. Acute GVHD is an acute inflammatory process that may involve skin, gastrointestinal tract and liver. Severe gastrointestinal GVHD may result in prolonged hospitalization from large volume diarrhea and gastrointestinal bleeding and possibly death. Staging and grading will be performed according to Appendix I. Chronic GVHD may involve skin, liver and lung and if severe may result in organ failure. An oral/ocular sicca syndrome is also commonly observed in patients with chronic GVHD. Chronic GVHD will be staged according to the NIH criteria (Appendix H). Patients with acute and chronic GVHD are treated with high-dose glucocorticoids and are at risk of opportunistic infections and other complications associated with steroid use.

Luznik et al (Blood 2010) reported that in a study of post-transplant high-dose cyclophosphamide after HLA-matched transplants from related or unrelated donors (n=117), the day-100 cumulative incidences of grades II through IV and III through IV acute GVHD for all patients were 43% (95% CI, 34%-52%) and 10% (95% CI, 6%-17%), respectively using only post-transplant high-dose cyclophosphamide for GVHD prevention [34]. Among recipients of grafts from related donors, the cumulative incidence of grades II through IV acute GVHD at day 100 was 42% (95% CI, 30%-52%), and for recipients of unrelated donor grafts the incidence was 46% (95% CI, 30%-61%). This difference was not significant (HR 0.87, 95% CI, 0.50-1.54, P=0.64). The cumulative incidences of grades III through IV acute GVHD at day 100 were 12% (95% CI, 6%-20%) and 8% (95% CI, 2%-19%) for recipients of related and unrelated donor grafts, respectively (HR 1.56, 95% CI, 0.43-5.62, P=0.50. This incidence of acute GVHD is mildly decreased from that the expected incidence of acute GVHD using a calcineurin inhibitor and methotrexate or MMF. It is expected that adding the combination of tacrolimus and MMF or Myfortic after high-dose cyclophosphamide will further decrease the risk of acute GVHD and the need for glucocorticoid treatment or additional immunosuppressive therapy.

With a median follow-up of surviving patients of 26.3 months (range, 10-55 months), the cumulative incidence of chronic GVHD for all patients was 10% (95% CI, 5%-16%). At 2 years after transplantation, the cumulative incidences of chronic GVHD for recipients of related and unrelated donor grafts were 9% (95% CI, 4%-17%) and 11% (95% CI, 3%-25%), respectively (HR 0.83, 95% CI, 0.25-2.88, P=0.79). Only 3 new cases occurred beyond 6 months after transplantation. Of 11 patients with chronic GVHD, 7 had classic limited and 3 had classic extensive forms of the disease. According to the National Institutes of Health severity scale, 3 had severe disease (2 with obliterative bronchiolitis). A total of 3 patients with chronic GVHD, remained on systemic immunosuppression at the time of last follow-up. The observed incidence of chronic GVHD after high-dose cyclophosphamide was markedly decreased compared to the expected incidence with standard GVHD prevention using calcineurin inhibitor and methotrexate or MMF.

Graft rejection will be defined as evidence that donor chimerism in CD33+ and CD3+ cells are <2%. GG: Earlier you wrote 5%--see section 4, Secondary endpoints, #8). After a standard-dose conditioning regimen of busulfan and cyclophosphamide, graft rejection was observed in 4.3% of cases [34]. The use of marrow rather than peripheral blood stem cells likely increased the risk of graft rejection but importantly may have contributed to the low risk of chronic GVHD. The proposed reduced-intensity regimen has been successfully used for HLA-haploidentical transplantation so it is expected that this regimen will successfully prevent rejection of HLA-identical grafts. The addition of tacrolimus and Myfortic or MMF after transplant is also expected to reduce the risk of graft rejection. Pretransplant treatment with pulse cyclophosphamide to treat and stabilize CD may precondition patients and further lower the risk of graft rejection. Since a reduced-intensity conditioning regimen is being used, if graft rejection occurs, recovery of autologous hematopoiesis is expected although there may be a prolonged period or pancytopenia before counts recover. This may result in severe or fatal infections. Second transplants have been performed successfully after graft rejection [105]. Patients who experience protracted neutropenia caused by graft rejection, will be reviewed by the FHCRC Patient Care Conference faculty where a decision will be made about a second transplant with autologous cells.

To manage the risk of opportunistic infections, patients will be monitored closely and receive preventative therapy according to **Section 10.8**.

13.0 GUIDELINES FOR ADVERSE EVENT REPORTING

13.1 Adverse Event Reporting/Institutional Policy

For a complete statement of institutional policy, see Unanticipated Problems Involving Risks to Subjects or Others IRB Policy 2.6 on the FHCRC IRO/IRB website at http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html. In accordance with institutional policy, all adverse events which in the opinion of the principal investigator are unexpected and related or possibly related to the research and serious or suggest that the research places research participants or others at greater risk of physical or psychological harm than was previously known or recognized be reported to the IRB within 10 calendar days of learning of the problem.

13.2 Definitions:

Adverse Event - Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, medical treatment or procedure and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, medical treatment or procedure whether or not considered related to the medicinal product.

Life-threatening Adverse Event – Any adverse event that places the patient or subject, in view of the investigator, at immediate risk of death from the reaction.

Unexpected Adverse Event – An adverse event is "unexpected" when its nature (specificity), severity, or frequency are not consistent with (a) the known or foreseeable risk of adverse events associated with the research procedures described in the Protocol-related documents, such as the IRB-approved research protocol,

informed consent document and other relevant sources of information such as product labeling and package inserts; and are also not consistent with (b) the characteristics of the subject population being studied including the expected natural progression of any underlying disease, disorder or condition any predisposing risk factor profile for the adverse event.

A listing of expected AEs for the subject population is included in Appendix J.

Serious Adverse Event (SAE) – Any adverse event occurring that results in any of the following outcomes:

- death
- a life-threatening adverse event (real risk of dying)
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly
- requires intervention to prevent permanent impairment of damage

Hospitalization is a part of the treatment plan and will not be considered an SAE unless it is unexpected or the duration of the hospital stay is unexpected.

Attribution - The following are definitions for determining whether an adverse event is related to a medical product, treatment or procedure:

- An adverse event is "**related or possibly related to the research procedures** "if in the opinion of the principal investigator, it was more likely than not caused by the research procedures.

- Adverse events that are solely caused by an underlying disease, disorder or condition of the subject or by other circumstances unrelated to either the research or any underlying disease, disorder or condition of the subject are not "related or possibly related."

- If there is any question whether or not an adverse event is related or possibly related, the adverse event should be reported.

The Cancer Consortium Expedited Reporting Form should be completed for all adverse events that meet the expedited reporting requirements. The AE form will be faxed to the IRO at (206) 667-6831. All available information should be submitted.

13.3. Safety Monitoring and Reporting

13.3.1 Overview

Safety data will be recorded on case report forms (CRFs) specifically designed for this purpose. All safety data will be reviewed monthly by the principal investigators and at least annually by the Data Safety and Monitoring Board (DSMB). The DSMB can recommend that any participant be withdrawn from the study and/or that the study be terminated because of safety findings.

13.3.2 Adverse Event Grading

Adverse Events will be graded according to the current version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4. The full text of the NCI CTCAE is available online at: http://evs.nci.nih.gov/ftp1/CTCAE/About.html

13.3.3 FHCRC IRB policies and forms for reportable events

All reportable adverse events will be collected and reported according to the FHCRC reporting guidelines. Definitions, instructions and forms associated with reportable events can be found in Appendix J and on the FHCRC's Institutional Review Office (IRO) extranet website: http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html.

Additional information about non-compliance and unanticipated problems can be found on the FHCRC's IRB Policy and Procedures extranet website: http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html.

13.3.4 Tracking of Events

Adverse events will be collected from the time the subject starts mobilization until day +365 after allogeneic HCT or until he/she prematurely withdraws from the study. From the start of mobilization of autologous stem cells until

day +100 after allogeneic HCT, grades 3, 4 and 5 adverse events will be captured. There are some exceptions that are outlined in Appendix J. Grades 3 and 4 adverse events in organs involved with CD will be reported only if they represent a significant change from baseline (pre-transplant) status.

By day +100, it is expected that patients will have recovered from the regimen-related toxicities associated with the conditioning regimen. Therefore only Grades 4 and 5 will be reported from day +100 through day +365 after transplant. After day +365, patients will be followed for CD and late sequella of allogeneic transplant. All deaths that occur throughout the course of the study, regardless of cause, will be reported to the IRB. Those occurring prior to year 1 will be reported in an expedited fashion and those occurring after 1 year will be reported with the annual renewal.

There are some categories of adverse events that will not be considered reportable events due to the lack of relevance to the evaluation of the protocol treatment. (see Appendix J). Certain adverse events are expected after high-dose immunosuppressive therapy and allogeneic HCT and are not informative and therefore will only be reported if they are $\geq$ Gr.4 from the start of mobilization until day +28. During this same period, there are some Gr. 3 and 4 adverse events that will not be reported at all. (see Appendix J). From day +29 until day +100, some grades 3 and 4 adverse events of the blood and lymphatic system will not be reported (see Appendix J).

14.0 ASSESSMENT OF DISEASE RESPONSE

14.1. Definition of study end-points and disease responses

Primary endpoint: event-free survival, defined as alive and free of active CD at 1 year after transplant.

Active CD: defined as abnormal mucosal inflammation characteristic of CD identified by endoscopy and biopsy of the gastrointestinal tract, or, if the area of intestinal inflammation cannot be reached with an endoscopy, identification will be by imaging such as CT or ultrasound or barium contrast study. The severity of CD will be scored using the CDAI (Appendix A) and the extent and severity of intestinal inflammatory lesions using SES-CD (Appendix C).

Event-Free Survival. Event-free is defined as for the primary endpoint. Patients alive at the time of last contact are censored.

Overall Survival: Death due to any cause is the event for this endpoint. Patients alive at the time of last contact are censored.

Treatment-related mortality: TRM is defined as death occurring at any time after start of allogeneic HCT and definitely or probably resulting from treatment given in the study and not associated with other unrelated causes.

14.1. Quality of life instruments

Because CD is a disorder that causes considerable morbidity, this protocol will include a quality of life instrument developed and validated in cohorts of patients with inflammatory bowel disease. The instrument to be used is the Short Inflammatory Bowel Disease Questionnaire (SIBDQ) (Appendix B), which incorporate elements of social, systemic and emotional symptoms, as well as bowel related symptoms into an activity index [25,26]. The SIBDQ will be administered at the following times: at baseline (before the start of conditioning therapy) and at intervals after transplant, as outlined in section 11.

15.0 DATA AND SAFETY MONITORING PLAN

Institutional support of trial monitoring is provided in accordance with the FHCRC Data and Safety Monitoring Plan (DSMP). The PDMC reviews accrual, serious adverse events, stopping rules and adherence to the protocol-specific data and safety monitoring plan. Under the provisions of the DSMP, the Clinical Research Support office provides monitoring for quality process and compliance by qualified monitors unaffiliated with the conduct of the study. Monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of the previous visit. The scope of monitoring is specified in the DSMP http://www.cancerconsortium.org/rto/prr/

Monitoring during the progress of the trial:
This clinical trial will be a single institution clinical trial that is monitored by the principal investigator (PI), the FHCRC Protocol and Data Monitoring Committee (PDMC) and the Institutional Review Board (IRB). The PI will review outcome data with the sub-investigators during scheduled monthly meetings or at a minimum, quarterly. Adverse events are reviewed with the PI on an ongoing basis in weekly meetings. The adverse event reporting in this clinical trial will follow the FHCRC Guidelines for adverse event reporting. For patients enrolled on this protocol but not being cared for at FHCRC, the outside facilities communicate with the PI and study coordinator for these reporting purposes. The PI for this study is responsible for adverse event reporting and summarizing reports of the toxicities with the annual renewal. The stopping rules provide an additional safeguard for adverse event analysis and reporting in this protocol. All collaborating investigators on the protocol will have fulfilled all NIH requirements for training in human subjects' protection.

All patients on study will be considered in the safety analysis. Because this is a phase 2 study, regimen related toxicities (RRT) and transplant related mortality (TRM) will be closely monitored. Aggregate study data will be evaluated to identify trends in adverse events.

Gastroenterologists that are co-investigators on this study will evaluate the disease responses. The potential safety and efficacy of the allograft approach will be evaluated by follow-up of patients over 5 years from the time of transplant.

Data Safety Monitoring Board
A Data Safety Monitoring Board (DSMB) will be constituted for this study and be composed of two CD experts and two experts in allogeneic hematopoietic cell transplantation. The PI for this study is responsible for adverse event reporting and summarizing reports of the toxicities. The stopping rules provide an additional safeguard for adverse event analysis and reporting in this protocol. They will review a listing of reported AEs and SAEs at least semi-annually (see Appendix J). The DSMB will review any events as requested by the investigators or the monitors. Further, the DSMB will be informed of all SAEs that are determined to warrant an expedited safety report. All deaths of patients enrolled in the protocol will be reviewed by the DSMB (see Section 17). If there is one episode of graft rejection with the first 5 patients on this protocol, a meeting of the DSMB will be convened to review potential changes to the protocol to prevent further episodes of graft rejection. After each DSMB meeting, recommendations will be forwarded to the investigators and to the FHCRC IRB.

16.0 DATA MANAGEMENT/CONFIDENTIALITY

Clinical Statistics maintains a subject database at FHCRC to allow storage and retrieval of subject data collected from a wide variety of sources. The investigator will ensure that data collected conform to all established guidelines for coding, collection, key entry and verification. Each subject is assigned a unique patient number to assure subject confidentiality. Subjects will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents. Subject research files are scanned and stored in a secure database (OWL). OWL records are maintained by the FHCRC data abstraction staff. Access is restricted to personnel authorized by the Division of Clinical Research.

17.0 STATISTICAL CONSIDERATIONS

This is a single-arm Phase II study with the objective of demonstrating the safety and efficacy of allogeneic HCT in treatment-refractory CD. The proposed sample size for this protocol is 12 subjects. The primary endpoint will be event-free survival at 1 year after transplant, defined as alive and free of active CD. Secondary endpoints will include event-free survival at 2 through 5 years; complete response rate at 1-5 years (CR defined as alive and free of active CD without immunosuppressive drugs); adverse events; frequency and severity of GVHD; and patient-reported outcomes.

No formal power analyses were conducted, since the study objectives require no hypothesis testing but rather descriptive statistics. The study sample size of 12 should allow a description of the rate of event-free survival with an exact 90% confidence interval that is less than ± 0.24 relative to the point estimate. An outcome of no disease

relapses in the 12 patients will allow us reasonable confidence (86%) that the true rate of relapse associated with this treatment is less than 15%.

The probability of event-free survival up to 5 years after transplant will be described graphically using a Kaplan-Meier estimate. An estimate of EFS at 1 year will be generated with confidence intervals using Greenwood's formula to calculate the standard error. The secondary outcomes will be characterized by the event rates as functions of all patients enrolled and at risk of the event, with exact confidence intervals.

The occurrence of one death of an enrolled patient will trigger a temporary cessation of the protocol. If a death occurs, no additional patients will be screened or consented or have the transplant procedure started until the death is reviewed by the Fred Hutchinson Cancer Research Center's Institutional Review Organization, the Protocol #2551 Data Safety and Monitoring Board, and FDA. The possible outcomes of this review process are a) temporary cessation of patient screening, consenting, and starting the transplant procedure while additional informative data is being collected; b) resumption of patient screening, consenting, and starting the transplant procedure, using the same protocol; c) resumption of patient screening and consenting under the aegis of a revised protocol, to be approved by the Fred Hutchinson Cancer Research Center IRO, the Protocol #2551 Data Safety and Monitoring Board, and FDA; or d) permanent closure of the protocol to enrollment.

A stopping rule will be imposed for transplant-related mortality (TRM) occurring within one year of transplant. The study will be stopped if at any point there is moderately strong evidence that the rate of TRM exceeds 10%. Moderately strong evidence will be taken to mean that the lower bound of a one-sided 80% confidence interval for the true rate of TRM is above 10%. Operationally, this criterion will be met if 2 deaths are observed within the first 8 patients or 3 deaths observed in 9-12 patients. The table below summarizes the operating characteristics of this stopping rule, where the probability of stopping the trial is estimated from 10,000 Monte Carlo simulations. Should the study be completed without stopping, it is recognized that an estimate of the rate of TRM will be biased downward. To the extent that various toxicities are correlated with mortality, estimates for the rates of these may also be biased downward.

Table 4. Probability of stopping the trial for excess transplant-related deaths.

Number of patients	True Rate of TRM	Probability of Stopping
6	10%	0.11
9	10%	0.13
12	10%	0.16
6	20%	0.35
9	20%	0.39
12	20%	0.51
6	30%	0.58
9	30%	0.65
12	30%	0.79

Table 5. Projected Target Accrual (ETHNIC AND GENDER DISTRIBUTION CHART)

TARGETED / PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	Sex / Gender		
	Females	Males	Total
Hispanic or Latino	1	1	2
Not Hispanic or Latino	5	5	10
Ethnic Category Total of All Subjects*	6	6	12
Racial Categories			

American Indian / Alaska Native	0	0	0
Asian	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	1	1	2
White	5	5	10
Racial Categories: Total of All Subjects*	6	6	12

18.0 TERMINATION OF STUDY

The PI may terminate the study at any time. The IRB also has the authority to terminate the study should it be deemed necessary.

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APPENDICES

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APPENDIX A

Crohn's Disease Activity Index for adult patients

Variables, severity scale, and weighting

Variable	Scale of Severity	Weight
Liquid or very soft stools	Number of stools summed daily for 7 days	2
Abdominal pain	Sum of 7 days of daily ratings, where 0 = none, 1 = mild, 2 = moderate and 3 = severe	5
General well-being	Sum of 7 days of daily ratings, where 0 = generally well, 1 = slightly below par, 2 = poor, 3 = very poor and 4 = terrible	7
Features of extraintestinal disease	Any of the following present during the recent 7 day period: •arthritis or arthralgia •skin or mouth lesions, including pyoderma gangrenosum, erythema nodsum, aphthous stomatitis •iritis or uveitis •anal fissure, fistula, or perirectal abscess •other external fistula •fever >100 degree F.	20 each
Opiates for diarrhea	0 = no, 1 = yes	30
Abdominal mass	0 = none, 2 = questionable, 5 = definite	10
Hematocrit value	Males, 47 minus hematocrit; females, 42 minus hematocrit	6
% body weight below standard	100 x (1 minus body weight/standard weight)	1

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Harvey-Bradshaw Index

1. General well-being (yesterday)	☐ Very well = 0 ☐ Slightly below par = 1 ☐ Poor = 2 ☐ Very poor = 3 ☐ Terrible = 4
2. Abdominal pain (yesterday)	☐ None = 0 ☐ Mild = 1 ☐ Moderate = 2 ☐ Severe = 3
3. Number of liquid or soft stools per day (yesterday)	(give number)
4. Abdominal mass	☐ None = 0 ☐ Dubious = 1 ☐ Definite = 2 ☐ Definite and tender = 3
5. Complications (check any that apply; score one per item except for "None")	☐ None ☐ Arthralgia ☐ Uveitis ☐ Erythema nodosum ☐ Aphthous ulcers ☐ Pyoderma gangrenosum ☐ Anal fissure ☐ New fistula ☐ Abscess
HARVEY-BRADSHAW INDEX SCORE (add scores of questions 1 through 5)	**HBI score = _____** **Remission <5** **Mild disease 5 – 7** **Moderate disease 8 – 16** **Severe disease >16**

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APPENDIX B

Short Inflammatory Bowel Disease Questionnaire—Quality of life instrument

This Evaluation is designed to find out how you have been feeling during the last 2 weeks. This can help us assess how much your disease is affecting your life. You will be asked about symptoms you are having as a result of your inflammatory bowel disease, the way you have been feeling in general, and how your mood has been. If you are uncomfortable answering any of these questions, you do not have to answer them.

1. **How often has the feeling of fatigue or of being tired and worn out been a problem for you during the last 2 weeks? Please indicate how often the feeling of fatigue or tiredness has been a problem for you during the last 2 weeks by picking one option from:**

1	All of the time
2	Most of the time
3	A good bit of the time
4	Some of the time
5	A little of the time
6	Hardly any of the time
7	None of the time

2. How often during the last 2 weeks have you had to delay or cancel a social engagement because of your bowel problem? Please choose an option from:

1	All of the time
2	Most of the time
3	A good bit of the time
4	Some of the time
5	A little of the time
6	Hardly any of the time
7	None of the time

3. How much difficulty have you had, as a result of your bowel problems, doing leisure or sports activities you would have like to have done over the last 2 weeks? Please choose an option from:

1	A great deal of difficulty, activities made impossible
2	A lot of difficulty
3	A fair bit of difficulty
4	Some difficulty
5	A little difficulty
6	Hardly any difficulty
7	No difficulty; the bowel problems did not limit sports or leisure activities

SUBTOTAL: _____

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4. How often during the last 2 weeks have you been troubled by pain in the abdomen? Please choose an option from:

1	All of the time
2	Most of the time
3	A good bit of the time
4	Some of the time
5	A little of the time
6	Hardly any of the time
7	None of the time

5. How often during the last 2 weeks have you felt depressed or discouraged? Please choose an option from:

1	All of the time
2	Most of the time
3	A good bit of the time
4	Some of the time
5	A little of the time
6	Hardly any of the time
7	None of the time

6. Overall, in the last 2 weeks, how much of a problem have you had passing large amounts of gas? Please choose an option from:

1	A major problem
2	A big problem
3	A significant problem
4	Some trouble
5	A little trouble
6	Hardly any trouble
7	No trouble

7. Overall, in the last 2 weeks, how much of a problem have you had maintaining or getting to the weight you would like to be? Please choose an option from:

1	A major problem
2	A big problem
3	A significant problem
4	Some trouble
5	A little trouble
6	Hardly any trouble
7	No trouble

SUBTOTAL: _____

8. How often during the last 2 weeks have you felt relaxed and free of tension? Please choose an option from:

1	None of the time
2	A little of the time
3	Some of the time
4	A good bit of the time
5	Most of the time
6	Almost all of the time
7	All of the time

9. How much of the time during the last 2 weeks have you been troubled by a feeling of having to go to the toilet even though your bowels were empty? Please choose an option from:

1	All of the time
2	Most of the time
3	A good bit of the time
4	Some of the time
5	A little of the time
6	Hardly any of the time
7	None of the time

10. How much of the time during the last 2 weeks have you felt angry as a result of your bowel problem? Please choose an option from:

1	All of the time
2	Most of the time
3	A good bit of the time
4	Some of the time
5	A little of the time
6	Hardly any of the time
7	None of the time

SUBTOTAL: _____

TOTAL SCORE: _____

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APPENDIX C

Simple Endoscopic Score for Crohn's Disease (SES-CD)

Variable	Simple Endoscopic Score (SES) for CD values, for each anatomic area			
	0	1	2	3
Size of ulcers	None	Aphthous ulcers, diameter 0.1 – 0.5 cm	Large ulcers, diameter 0.5 – 2 cm	Very large ulcers, diameter > 2 cm
Ulcerated surface (%)	None	<10%	10 – 30%	>30%
Affected surface (%)	Anatomic area unaffected	<50%	50 – 75%	>75%
Presence of narrowings	None	Single, can be passed with endoscope	Multiple, can be passed with endoscope	Cannot be passed with endoscope

SES-CD Scoring Form*

	Anatomic area examined							Total
	Stomach & duodenum	Jejunum	Ileum	Right colon	Transverse colon	Left Colon	Rectosigmoid Colon	
Presence and size of ulcers (0 – 3)								
Extent of ulcerated surface (0 – 3)								
Extent of affected surface (0 – 3)								
Presence and type of narrowings (0 – 3)								
							SES-CD	

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APPENDIX D

Crohn's Disease Detailed History

Patient Name			**FHCRC Unique Patient Number**	
Date of Birth			**University of Washington U-number**	
Age at onset of CD symptoms (year)				
SURGICAL HISTORY			**MEDICATION HISTORY**	
Number of surgical procedures for CD			**Number of years on CD medications**	
Year of 1st surgery and description			**Check list of CD medications—check the appropriate box if any medications in this category have been taken at any time in the past**	☐ Aminosalicylate ☐ Antibiotic ☐ Budesonide ☐ Azathioprine ☐ 6-mercaptopurine ☐ 6-thioguanine ☐ Methotrexate ☐ Cyclophosphamide ☐ Calcineurin inhibitor ☐ Prednisone ☐ Anti-TNFα ☐ Anti-integrin ☐ Another Rx (list)
Year of 2nd surgery and description			**Number of different medical treatment regimens in the last 12 months (list)**	
Year of 3rd surgery and description			**Current medications for CD and its symptoms (list)**	
Year of 4th surgery and description			**Current medications for other conditions (list)**	
Year of 5th surgery and description				
CURRENT ANATOMY OF THE GASTROINTESTINAL TRACT				

Current anatomy and CD activity: Esophagus	☐ Anatomy normal ☐ Anatomy abnormal (describe) ☐ Inflammation absent ☐ Inflammation present (describe)		**Current anatomy: stomach**	☐ Anatomy normal ☐ Anatomy abnormal (describe) ☐ Inflammation absent ☐ Inflammation present (describe)
Current anatomy: duodenum	☐ Anatomy normal ☐ Anatomy abnormal (describe) ☐ Inflammation absent ☐ Inflammation present (describe)		**Current anatomy: jejunum-ileum**	☐ Anatomy normal ☐ Anatomy abnormal (describe) ☐ Inflammation absent ☐ Inflammation present (describe)
Current anatomy: colon	☐ Anatomy normal ☐ Anatomy abnormal (describe) ☐ Inflammation absent ☐ Inflammation present (describe)		**Current anatomy: anorectal**	☐ Anatomy normal ☐ Anatomy abnormal (describe) ☐ Inflammation absent ☐ Inflammation present (describe)
Current extra-intestinal manifestations of CD	☐ Skin ☐ Liver ☐ Eyes ☐ Lungs ☐ Other site(s) (list)		**Current laboratory abnormalities related to CD**	
WEIGHT AND HEIGHT				
Body weight **•current body weight in kg** **•maximum body**	_____ kg		**Current height (in both meters and centimeters)**	_____ cm _____ m

weight (at this approximate age) **•minimum body weight (at this approximate age)**	_____ kg (@ age ___) _____ kg (@ age ___)			
Adjusted Ideal Body Weight	_____ kg		**Body Mass Index (weight in kg / height in meters)**	

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APPENDIX E

Eligibility Checklist

Inclusion criteria (all must be checked)	Exclusion criteria (none can be checked)
☐ Certain diagnosis of Crohn's Disease	
Adverse prognosis as indicated by treatment-refractory gastrointestinal mucosal inflammation, and documented by persistent signs and symptoms of CD that have failed to respond to medical and surgical therapies, including the use of systemic immune suppressive drugs and biopharmaceutical(s). Refer to Section 5.1, paragraph 2 (a through f) for details of past therapies that constitute the criteria for refractoriness to treatment, of which the following is a summary: ☐ Systemic glucocorticoids (see Section 5.1) ☐ Methotrexate and/or a thiopurine antimetabolite (see Section 5.1). If a patient is homozygous mutant for the TPMT gene, thiopurines would be contraindicated and their use would not be a requirement for enrollment in this protocol. ☐ Use of at least two anti-TNF-alpha therapies (infliximab and/or adalimumab and/or certolizumab pegol. ☐ Exhaustive surgical treatment will be defined as indicated operations for complications of Crohn's Disease up to the point where the risks of surgery are deemed by patients and their physicians to be unacceptably high. Indicated operations for complications of Crohn's Disease include, but are not limited to, surgical resection of involved intestine, stricturoplasty, drainage, curettage, or adhesiolysis of tissues affected by Crohn's disease).	☐ Current complication of CD that would jeopardize survival: ☐ Abscess, phlegmon, necrotizing ☐ Intestinal fibrotic stricture or intestinal obstruction ☐ Infection ☐ Sclerosing cholangitis ☐ Other
☐ Endoscopic/histologic or imaging evidence of current intestinal inflammation	☐ History of Progressive Multifocal Leukoencephalopathy
☐ Severe Crohn's Disease as defined by one of the following: ☐ CDAI >250 ☐ Need for TPN ☐ Recurrent inflammation after surgical resection	☐ Organ dysfunction or disease that would jeopardize survival ☐ Renal insufficiency ☐ Cardiac dysfunction ☐ Significant pulmonary dysfunction ☐ Necroinflammatory/fibrotic liver dis. ☐ Marrow dysfunction ☐ Poorly controlled hypertension ☐ Neurologic dysfunction ☐ Poorly controlled diabetes mellitus ☐ Extreme protein-calorie malnutrition
☐ HLA-matched donor negative for a history of or signs and symptoms consistent with inflammatory bowel disease, a serious autoimmune disease, or another serious disorder that can be passaged by bone marrow transplantation, and	☐ Pregnancy ☐ Unwillingness to practice contraception

without NOD2 mutation (in the case of an HLA-matched sibling). **OR** ☐ HLA-matched unrelated donor negative for a history of or signs and symptoms consistent with inflammatory bowel disease, a serious autoimmune disease, or another serious disorder that can be passaged by bone marrow transplantation as determined by NMDP screening of donor medical issues.	☐ History of smoking within 3 months ☐ HIV, HBV, or HCV seropositive ☐ Short life expectancy ☐ Psychiatric illness ☐ Inability to consent ☐ Prior lack of compliance ☐ History of malignancy other than skin ☐ HCT-CI >2 (adults only)
☐ Age between 18 and 60	

Study Investigator's Signature _____ Date _____

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APPENDIX F

Hematopoietic cell transplant co-morbidity index (HCT-CI)

Patient Name_____ U-number _____UPN_____ Date_____

Assign scores appropriately if the patient has any of these comorbidities

Comorbidity	Definition / compartments	Yes	Score
1. Arrhythmia	– Atrial fibrillation*	☐	
	– Atrial flutter*	☐	1
	– Sick sinus syndrome*	☐	
	– Ventricular arrhythmia*	☐	
2. Cardiovascular	– Coronary artery disease*	☐	
	– Congestive heart failure*	☐	1
	– Myocardial infarction*	☐	
	– Ejection fraction ≤ 50%§	☐	
3. Diabetes	– Rx with insulin or oral hypoglycemic drugs*	☐	1
4. Cerebro-vascular	– Transient ischemic attacks*	☐	☐
	– Ischemic or hemorrhagic stroke*	☐	
5. Depression / anxiety	– Requiring psych consult or specific Rx§	☐	1
6. Hepatic – mild	– Chronic hepatitis§	☐	
	– Total serum bilirubin >ULN to 1.5 X ULN§	☐	1
	– AST/ALT >ULN to 2.5XULN§	☐	
7. Obesity	– Body mass index >35 (adults)§	☐	1
	– BMI-for-age ≥95th percentile (children)§	☐	
8. Infection	– Requiring anti-microbial treatment before, during, and after the start of conditioning regimen§	☐	1
9. Rheumatologic	– Required treatment*	☐☐☐	2
10. Peptic ulcer	– Confirmed by endoscopy and required Rx*	☐☐☐	2
11. Renal	– Serum creatinine > 2mg/dL (or >177 µmol/L)§	☐	
	– On dialysis§	☐	2
	– Prior renal transplantation*	☐	
12. Pulmonary – Moderate	– DLco corrected for Hgb 66-80% of predicted§	☐☐☐	
	– FEV_1 66-80% of predicted§	☐	2
	– Dyspnea on slight activity§	☐☐☐	

13. Pulmonary – Severe	— DLco corrected for Hgb ≤ 65% of predicted§ — FEV_1 ≤ 65% of predicted§ — Dyspnea at rest or requiring O_2 therapy§	☐ ☐ ☐	3
14. Heart valve disease	— Except asymptomatic mitral valve prolapse§	☐	3
15. Prior malignancy	— Treated with surgery, chemotherapy, and/or radiotherapy excluding non-melanoma skin cancer*	☐	3
16. Hepatic – moderate/severe	— Liver cirrhosis§ — Total serum bilirubin >1.5 X ULN§ — AST/ALT >2.5XULN§	☐ ☐ ☐	3

Total score = ____

* Diagnosed at any time in the patient's past history.
§ Detected at the time of pretransplant assessment.
Abbreviations: ULN indicates upper limit of normal; DLco, diffusion capacity of carbon monoxide; FEV_1, forced expiratory volume in one second; AST, aspartate aminotransferase; and ALT, alanine aminotransferase.

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APPENDIX G

Study Calendar

Study Evaluations	During 1st and 2nd Phase Screening Visits	During time from transplant consent to start of conditioning therapy	Days post-transplant				Long Term Follow-up
			Day 0	Daily to engraftment	Weekly post engraftment to Day 80	Day 80 ± 5 days	To years 1, 2, 3, 4, 5
Informed Consent	X	X					
Medical History, Physical Exam	X	X	X	X	X	X	X
CBC, blood chemistries	X	X	X	X	X	X	X
Urine collection	X					X	X
Pregnancy test	X	X					
Body Mass Index	X					X	X
Fertility counseling	X						
Dental evaluation	X						
CDAI	X					X	X
SIBDQ (QOL)	X					X	X
HCT-Comorbidity Index	X						
Endoscopic evaluation	X					X	X (years 1, 3 and 5)
Magnetic resonance imaging, CT enterography or a comparable intestinal imaging method	X					(X)	X (year 1)
Pulmonary tests	X					X	X (year 1)
Cardiac tests	X						
Stool specimen	X						
Viral serologies	X						
Research blood samples	X					X	X
Respiratory virus screening		X					

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EBV DNA by PCR		X				X	Per FHCRC Standard Practice
Hickman catheter placement		X					
Harvest of autologous PBSCs		X					
CMV DNA Surveillance by PCR		X	Once weekly from Day 0 until Day 100				Per FHCRC Standard Practice
Chimerism – Peripheral Blood						X	X
IgA, IgG, IgM levels						X	X
Acute and chronic GVHD evaluation				X	X	X	X
Vaccine antibody titers						X	X

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APPENDIX H

Chronic graft-versus-host disease grading

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* ☐ Maculopapular rash ☐ Lichen planus-like features ☐ Papulosquamous lesions or ichthyosis ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Keratosis pilaris ☐ Erythema ☐ Erythroderma ☐ Poikiloderma ☐ Sclerotic features Rodnan score: ☐ ☐ Pruritus ☐ Hair involvement ☐ Nail involvement **% BSA involved** ☐	☐ No Symptoms	☐ <18% BSA with disease signs but **NO** sclerotic features	☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐ >50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
☐ *Abnormality present but NOT thought to represent GVHD*				
MOUTH *Diagnosti/distinctive features* ☐ Present ☐ Absent	☐ No symptoms	☐ Mild symptoms with disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
☐ *Abnormality present but NOT thought to represent GVHD*				
EYES Mean tear test (mm): ☐ >10 ☐ 6-10 ☐ ≤5 ☐ Not done	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	☐ Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), **WITHOUT** vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitis sicca
☐ *Abnormality present but NOT thought to represent GVHD*				
GI TRACT	☐ No symptoms	☐ Symptoms such as nausea, vomiting,anorexia,dysphagia, abdominal pain or diarrhea without significant weight loss (<5%)	☐ Symptoms associated with mild to moderate weight loss (5-15%)	☐ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation
☐ *Abnormality present but NOT thought to represent GVHD*				

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	SCORE 0	SCORE 1	SCORE 2	SCORE 3
LIVER	☐ Normal LFT	☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN	☐ Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN	☐ Bilirubin or enzymes > 5 x ULN
☐ *Abnormality present but NOT thought to represent GVHD*				
LUNGS‡	☐ No symptoms	☐ Mild symptoms (shortness of breath after climbing one flight of steps)	☐ Moderate symptoms (shortness of breath after walking on flat ground)	☐ Severe symptoms (shortness of breath at rest; requiring 0_2)
FEV1 ☐				
DLCO ☐	☐ FEV1 > 80% **OR** LFS=2	☐ FEV1 60-79% **OR** LFS 3-5	☐ FEV1 40-59% **OR** LFS 6-9	☐ FEV1 ≤39% **OR** LFS 10-12
☐ *Abnormality present but NOT thought to represent GVHD*				
JOINTS AND FASCIA	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
☐ *Abnormality present but NOT thought to represent GVHD*				
GENITAL TRACT *Diagnostic features:* ☐ Present ☐ Absent ☐ Not examined	☐ No symptoms	☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	☐ Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum
☐ *Abnormality present but NOT thought to represent GVHD*				

Other indicators, clinical manifestations or complications related to chronic GVHD (check all that apply):

☐ Weight loss	☐ Bronchiolitis obliterans	☐ Bronchiolitis obliterans with organizing pneumonia	
☐ Esophageal stricture or web (serositis)	☐ Pericardial Effusion	☐ Pleural Effusion(s)	☐ Ascites
☐ Nephrotic syndrome Polymyositis	☐ Peripheral Neuropathy	☐ Myasthenia Gravis	☐
☐ Malabsorption Cardiomyopathy	☐ Cardiac conduction defects	☐ Coronary artery involvement	☐
☐ Eosinophilia >500/microliter	☐ Other: _____		☐ **None**

Biopsy obtained: ☐**Yes** ☐**No** **Organ system(s) biopsied:**_____ **GVHD confirmed by histology:** ☐**Yes** ☐**No**

OVERALL severity of GVHD: ☐ **None** ☐ **Mild** ☐ **Moderate** ☐ **Severe**

Change from previous evaluation: ☐ **None** ☐ **Improved** ☐ **Stable** ☐ **Worse** ☐ **N/A (baseline)**

Completed by:_____ **Date form completed:**_____

Pulmonary scoring should be performed using both the symptom and pulmonary function testing (PFT) scale whenever possible. When discrepancy exists between pulmonary symptom or PFT scores the higher value should be used for final scoring. Scoring using the Lung Function Score (LFS) is preferred, but if DLCO (carbon monoxide diffusion capacity corrected for hemoglobin) is not available, grading using FEV1 (forced expiratory volume) should be used. The LFS is a global assessment of lung function after the diagnosis of bronchiolitis obliterans has already been established.[28] The percent predicted FEV1 and DLCO (adjusted for hematocrit but not alveolar volume) should be converted to a numeric score as follows: > 80% = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; < 40% = 6. The LFS = FEV1 score + DLCO score, with a possible range of 2-12.

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APPENDIX I

Acute GVHD Grading Scale

Severity of Individual Organ Involvement			
System	Severity		
Skin	☐	+1	maculopapular eruption involving less than 25% of the body surface
	☐	+2	maculopapular eruption involving 25-50% of the body surface
	☐	+3	generalized erythroderma
	☐	+4	generalized erythroderma with bullous formation + desquamation
Liver	☐	+1	bilirubin (2.0-3.0 mg/100 ml)
	☐	+2	bilirubin (3-5.9 mg/100 ml)
	☐	+3	bilirubin (6-14.9 mg/100 ml)
	☐	+4	bilirubin > 15 mg/100 ml
Gut	☐	+1	≤ 1000 ml of liquid stool/day* (≤ 15ml of stool/kg/day)†
	☐	+1	Nausea or vomiting or anorexia
	☐	+2	>1,000 ml of stool/day* (> 15ml of stool/kg/day)†
	☐	+3	>1,500 ml of stool/day* (> 20ml of stool/kg/day)†
	☐	+4	2,000 ml of stool/day* (≥ 25ml of stool/kg/day)
*In the absence of infectious/medical cause			

Overall Grade		(Maximum grade)
☐	I	+1 to +2 skin rash. No gut or liver involvement.
☐	II	+1 to +3 skin rash or
		+1 gastrointestinal involvement and/or +1 liver involvement
☐	III	+2 to +4 gastrointestinal involvement and/or
		+2 to +4 liver involvement with or without a rash
☐	IV	Pattern and severity of GVHD similar to grade 3 with extreme constitutional symptoms or death.

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APPENDIX J

Adverse events reporting guidelines for this protocol

Adverse Events in certain categories that are not considered as reportable adverse events.

The following events under certain categories will not be considered reportable adverse events due to the lack of relevance to the evaluation of the protocol treatment. Furthermore, in many cases, the event if significant will be captured more appropriately under another adverse event category (e.g. it is most relevant to report the event causing pain rather than the pain event itself):

- Congenital, familial and genetic disorders
- Endocrine disorders: puberty and growth related events
- General disorders and administration site conditions: chills, edema, pain, fatigue, fever, flu-like symptoms, irritability and malaise.
- Investigations: elevations in liver enzymes without increase in bilirubin, gonadotropin, prolactin, decreased lymphocyte and CD4 T cell count since this is expected with transplant, increase in amylase or lipase without clinical evidence of pancreatitis and weight gain or loss.
- Metabolism and nutrition disorders: anorexia.
- Musculoskeletal and connective tissue disorders: exostosis, generalized muscle weakness, growth suppression, change in joint range of motion, kyphosis, lordosis, myalgia, myositis, scoliosis, trismus and unequal leg length.
- Pain
- Pregnancy, puerperium and perinatal conditions: all events listed
- Psychiatric disorders: agitation, anxiety, euphoria, insomnia, change in libido and restlessness
- Reproductive system and breast disorders: all events listed
- Skin and subcutaneous tissue disorders: dry skin, fat atrophy, hyperhidrosis, lipohypertrophy and pruritis
- Social circumstances: both events listed
- Vascular disorders: flushing, hot flashes
- Surgical and medical procedures

Grading of adverse events from the start of mobilization until day +28.

1) Grade 3 and 4 adverse events that will not be reported during the first 28 days:
Certain Grade 3 and 4 adverse events under particular categories in the CTC are expected as part of treatment or not uncommon in the HCT setting and do not represent a life-threatening event as detailed. These adverse events will not be reported at any time during the first 28 days:

Anemia
Lymphocyte count decreased
Neutrophil count decreased
Platelet count decreased
White blood cell decreased
Fatigue
Fever
Anorexia
Insomnia
Chills
Weight gain
Weight loss
Vomiting
Hypokalemia
Hyponatremia
Hypomagnesemia
Hyperglycemia
Hypocalcemia
Hypophosphatemia

The primary hospitalization and re-hospitalizations to manage expected events will not be automatically reported.

2) Adverse events that will be reported only if ≥ Grade 4 during the first 28 days:
Since the following events are to be expected in the setting of HCT, they will only be reported if they are ≥ Grade 4 severity:

Blood and Lymphatic System Disorders
DIC
Thrombotic microangiopathy

Gastrointestinal Disorders
Colitis
Diarrhea associated with radiation or BMT studies
Dysphagia, esophagitis, odynophagia
Dysphagia-esophageal related to radiation
Dysphagia-pharyngeal related to radiation
Gastritis
GI bleeding
Ileus
Mucositis
Nausea
Pancreatitis
Stomatitis/pharyngitis/mucositis
Vomiting

Hepatobiliary disorders
Hepatic hemorrhage

Metabolism and Nutrition Disorders
Dehydration
Acidosis
Alkalosis
Hypercalcemia
Hyperkalemia
Hypermagnesemia
Hypernatremia
Hypertriglyceridemia
Hyperuricemia
Hypoglycemia

Infections
All infections

Investigations
Cholesterol high
Blood bilirubin increased
Creatinine increased

Renal and urinary disorders
Acute kidney injury
Bladder spasms
Cystitis noninfective
Hematuria
Renal hemorrhage
Urinary electrolyte wasting
Urinary frequency/urgency
Urinary retention

Reproductive system and breast disorders
Menorrhagia

Respiratory, thoracic and mediastinal disorders
Cough
Hemoptysis
Bronchopulmonary hemorrhage
Hypoxia
Laryngeal hemorrhage
Pharyngeal hemorrhage
Pleural hemorrhage
Pleural effusion
Pneumonitis/pulmonary infiltrates
Voice alteration/stridor

Vascular Disorders
Hypertension
Hypotension

Grading of adverse events from day +29 to day +100.

1) Grade 3 and 4 events that will not be reported:
- Blood and lymphatic system disorders except for DIC, TTP and HUS.
- Decreases in blood counts including white blood cells, neutrophils, lymphocytes, hemoglobin (anemia) and platelets.

2) All other grade 3 and 4 events will be reported

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APPENDIX K

PROTOCOL 2551: CROHN'S ALLOGENEIC TRANSPLANT STUDY

ELIGIBILITY REVIEW GROUP DOCUMENTATION

Patient Name:

U-number:

We reviewed the following to determine whether this patient potentially meets the eligibility criteria set forth in Protocol 2551, Allogeneic hematopoietic cell transplantation for patients with treatment-refractory Crohn's Disease: A phase 2 study.

- Patient-completed questionnaire (from www.CATS-FHCRC.org) dated _____
- Outside medical records
- Consultation notes from visit to Seattle Cancer Care Alliance dated _____
- Crohn's Disease Activity Index dated _____
- Appendix E (Eligibility check list) Review of available patient data after First Phase screening visit.

Eligibility determination (check one):

☐ This patient meets eligibility criteria at this time

☐ This patient does not meet eligibility criteria at this time

☐ This patient may meet eligibility criteria in the future provided that the following issue(s) are addressed:

_____
_____
_____

Comments:

Timothy L. Zisman, MD, MPH	George E. Georges, MD	George B. McDonald, MD
Date	Date	Date