Supplemental Methods

Product manufacturing and trial enrollment

Approval was obtained for an open label, Phase I prospective study of autologous MSC-coated fistula plugs in patients with fistulizing CD (Investigational New Drug (IND) #15356 and through the Mayo Clinic Institutional Review Board (IRB). Patients with CD ages 18-65, with a single draining fistula for at least 3 months despite medical therapy, without contraindication to Magnetic Resonance Imagining (MRI) evaluation, and who failed standard therapy including anti-TNF therapy were eligible. Patients were excluded if they had clinically significant comorbidities within 6 months of MSC harvest, history of cancer, hepatitis or HIV, or were pregnant or lactating.

Informed consent was obtained from all patients. Patients underwent a baseline general exam, and serologic studies including complete blood count (CBC) with differential, C reactive protein (CRP), erythrocyte sedimentation rate (ESR), and electrolytes. Patients were scheduled for an exam under anesthesia (EUA) to confirm the fistula tract and architecture, to drain sepsis if present, and to place a seton. At the time of this operation, a 2 cm transverse incision was made in the abdominal wall to obtain up to 4 grams of adipose tissue, collected under sterile conditions. Cells were processed in the Human Cell Therapy Lab under the guidance of the Mayo Clinic Division of Transfusion Medicine Quality program using approved Standard Operating Procedures (SOPs) in accordance with current Good Manufacturing Practices (GMPs)[1-4]. After obtaining sufficient cells to harvest and load the matrix, cells were cryo-preserved and samples were used for release testing consisting of phenotype (CD44,

CD73, CD105, Class I, CD14, CD45 and Class II), mycoplasma, culture sterility (aerobic and anaerobic), and cytogenetic analysis. When the patient was scheduled for plug placement, MSCs meeting release criteria were thawed and returned to culture in the presence of a Gore® Bio-A® Fistula Plug (MATRIX) in a polypropylene coated bioreactor for 3-6 days. Post thaw viability was calculated using trypan blue exclusion. Cell retention after cell administration to the plug was calculated by removing a sample of the supernatant, counting the cells, multiplying by the volume of media, and then expressed as a percentage of the cells delivered to the bioreactor. The resultant average dose was approximately $20x10^6$ cells per plug.

Prior to administration to the patient, the media used to incubate the cell/plug combination was evaluated with a gram stain and a sample sent for additional sterility testing. The plug was washed to remove unbound cells and media, and then maintained in lactated ringers until delivery for administration.

Patients underwent intraoperative placement of the stem cell loaded plug (MSC-MATRIX) approximately 6 weeks following the MSC harvest. The operation involved removal of the previously placed seton, curetting the fistula tract, and placement of the MSC-MATRIX fistula plug. The plug was passed through the tract and secured at the internal opening using 4 to 6 sutures. The external opening was widened appropriately to allow adequate drainage. To standardize the technical aspects of the surgical procedures, all operations were performed by the same surgeon (EJD). Patients were observed for 6 hours for acute adverse events before discharge from the hospital, and seen again in clinic the following day. Subsequent visits occurred at week 2, and 1, 2, 3, and 6 months following MSC-MATRIX placement at which time a clinical exam was

performed to 1) assess the opening of the fistula tract and 2) attempt to express any fluid from the fistula tract with deep palpation. MRI was performed prior to surgery and at Week 2, Month 2, and 6 months.

Conventional multiplanar, multisequence pelvic MRI using a torso-phased array coil for perianal fistula detection and characterization. A radiologist with 18 years of experience in reading pelvic MRI interpreted MRI images, and classified the fistulas according to the Park's and St. James classification systems. Fistula activity was characterized using the Van Assche score, which grades fistula activity according complexity, extension, T2 hyper-intensity and other complications[5]. Surrogate quantitative markers of fistula activity were also measured, including maximum fistula diameter and length of the hyperintense T2 tract. The length and diameter of T2-weighted hyperintensity within the fistula tract was chosen for measurement as T2-weighted hyperintensity within fistulas reflects fluid and granulation tissue, and decrease in fistula size and reduction in length is associated with fistula response[6]. All authors had access to the study data and reviewed and approved the final manuscript.

Evaluation of response to treatment

Primary Endpoint (Safety):

The primary endpoint of this study was to determine the safety and feasibility of using adipose derived, autologous MSC-MATRIX for treatment of refractory CD perianal fistulas. We monitored the subjects for the following adverse events:

1. Worsening (change in nature, severity, or frequency) of CD present at the time of the study

2. Intercurrent illnesses
3. Abnormal laboratory values (this includes clinically significant shifts from baseline within the range of normal that the investigator considers to be clinically significant).
4. Clinically significant abnormalities in physical examination, vital signs, weight, drainage for the perianal fistulas.

Secondary endpoint (Efficacy):

1. Clinical assessment of drainage on physical exam (Fistula closure was defined as the absence of drainage; spontaneous or with gentle compression) at the Week 24 (6 month) visit.
2. Radiographic response by MRI, the gold standard test for assessment of presence and activity[7]

For the purposes of this study, fistula activity was defined in 2 ways: 1) clinically and 2) radiographically. Clinically, a <u>partial response</u> was defined as decreased drainage and symptoms and a <u>complete response</u> was defined as complete cessation of drainage (some patients have a persistent skin defect preventing the use of the term “complete closure”). Radiographic response was defined by decrease in the diameter and length of the T2-weighted hyperintense fistula tract on T2-weighted fast spin-echo images (expressed as percentage change from baseline), without development of abscess or additional ramifications off the treated fistula, and without increase in the Van Aasche MRI perianal fistula severity score[5]. A decrease in the Van Aasche score was not

required for treatment response, as marked reductions in fistula size can be seen without changes in the Van Aasche score; however, any increase in Van Aasche was considered failure of response, as an increase in fistula ramifications or abscess would increase score components.

Growth kinetics, phenotype and characterization of cells used for therapy

The protocol proved highly feasible with every biopsy capable of generating a viable clinical stem cell product. One patient required re-collection of adipose tissue due to contamination and one due to failure to follow GMP. Cells grew rapidly with average doublings of 1.3 per day. Our protocol administered live, recently bound cells to a matrix. Release testing was done at the time of cryopreservation. Post thaw viability was routinely above 95%. Cells were counted in the supernatant during cell binding to properly understand the dose of cells on the matrix. For all samples, less than 5% (mean of 1.5%) of the cells remained in the supernatant on completion of the incubation, confirming their ability to recover and grow well following storage (Figure 1A). Patient MSCs universally demonstrated the classic MSC phenotype that is positive (>95%) for CD44, CD73, CD105 and MHC Class I, and negative (<5%) for CD14, CD45 and MHC Class II. Cell morphology at the time of collection is fibroblast-like and a representative example of MSC-MATRIX combined product prior to administration shows that cells remain viable.

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