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CLINICAL AT

# Allogeneic Bone Marrow—Derived Mesenchymal Stromal Cells Promote Healing of Refractory Perianal Fistulas in Patients With Crohn's Disease

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**BACKGROUND & AIMS:** Patients with perianal fistulizing Crohn's disease have a poor prognosis because these lesions do not heal well. We evaluated the effects of local administration of bone marrow–derived mesenchymal stromal cells (MSCs) to these patients from healthy donors in a double-blind, placebo-controlled study. **METHODS:** Twenty-one patients with refractory perianal fistulizing Crohn's disease were randomly assigned to groups given injections of $1 \times 10^7$ (n = 5, group 1), $3 \times 10^7$ (n = 5, group 2), or $9 \times 10^7$ (n = 5, group 3) MSCs, or placebo (solution with no cells, n = 6), into the wall of curettaged fistula, around the trimmed and closed internal opening. The primary outcome, fistula healing, was determined by physical examination 6, 12, and 24 weeks later; healing was defined as absence of discharge and <2 cm of fluid collection—the latter determined by magnetic resonance imaging at week 12. All procedures were performed at Leiden University Medical Center, The Netherlands, from June 2012 through July 2014. **RESULTS:** No adverse events were associated with local injection of any dose of MSCs. Healing at week 6 was observed in 3 patients in group 1 (60.0%), 4 patients in group 2 (80.0%), and 1 patient in group 3 (20.0%), vs 1 patient in the placebo group (16.7%) ($P = .08$ for group 2 vs placebo). At week 12, healing was observed in 2 patients in group 1 (40.0%), 4 patients in group 2 (80.0%), and 1 patient in group 3 (20.0%), vs 2 patients in the placebo group (33.3%); these effects were maintained until week 24 and even increased to 4 (80.0%) in group 1. At week six, 4 of 9 individual fistulas had healed in group 1 (44.4%), 6 of 7 had healed in group 2 (85.7%), and 2 of 7 had healed in group 3 (28.6%) vs 2 of 9 (22.2%) in the placebo group ($P = .04$ for group 2 vs placebo). At week twelve, 3 of 9 individual fistulas had healed in group 1 (33.3%), 6 of 7 had healed in group 2 (85.7%), 2 of 7 had healed in group 3 (28.6%), and 3 of 9 had healed in the placebo group (33.3%). These effects were stable through week 24 and even increased to 6 of 9 (66.7%) in group 1 ($P = .06$ group 2 vs placebo, weeks 12 and 24). **CONCLUSIONS:** Local administration of allogeneic MSCs was not associated with severe adverse events in patients with perianal fistulizing Crohn's disease. Injection of $3 \times 10^7$ MSCs appeared to promote healing of perianal fistulas. ClinicalTrials.gov ID NCT01144962.

*Keywords:* Cell Therapy; Perianal Fistulas; Treatment; Inflammatory Bowel Disease.

Perianal fistulizing Crohn's disease (CD) remains a significant clinical challenge greatly affecting patients' quality of life due to pain, discharge, and abscess formation.[1] At least 23%–26% of CD patients develop perianal fistulas within 20 years after diagnosis.[2,3] Achieving complete fistula healing is often a long process accompanied by multiple relapses. Patients frequently fail to respond to current medical options, including antibiotics, immunosuppressive agents, and anti-tumor necrosis factor (TNF) biologicals.[4–6] To prevent abscess formation, surgical placement of noncutting setons is often required. In more severe cases, fecal diversion is needed to attenuate perianal disease.[7] The ultimate treatment goal is complete fistula healing without sphincter damage. Unfortunately, despite the best available therapies, durable remission rates of complex perianal fistulas remain low at 37.0%.[8]

An emerging therapeutic approach is the use of mesenchymal stromal cells (MSCs). These are nonhematopoietic multipotent cells able to down-regulate immune responses and promote tissue healing. It has been reported that human MSCs are able to inhibit generation of dendritic cells from monocytes, are capable of down-regulating expression of

***Abbreviations used in this paper:*** **CD, Crohn's disease; CDAI, Crohn's Disease Activity Index; IL, interleukin; LUMC, Leiden University Medical Center; MRI, magnetic resonance imaging; MSC, mesenchymal stromal cell; PDAI, Perianal Disease Activity Index; TNF, tumor necrosis factor.**

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presentation and costimulatory molecules on mature dendritic cells preventing T-cell activation, and promote the generation of regulatory T cells.[9−14] In addition, MSCs participate in tissue-repair processes, providing a strong rationale for the use of these cells as a treatment for perianal fistulizing CD. Recently, phase I and II clinical trials have shown promising results on the healing rates of perianal fistulas.[15−20] Locally injected MSCs demonstrated 69%−82% fistula healing.[16−18,20] MSCs used in these trials were predominantly harvested from autologous adipose tissue and were reported to be a safe and feasible option. However, autologous MSCs are not immediately available upon request because isolation and expansion of MSCs to sufficient numbers of cells requires weeks, resulting in treatment delay. In addition, the possibility of disease-related effects on autologous MSCs must be taken into account. Therefore, we used allogeneic MSCs derived from bone marrow aspirates of healthy donors. Until now, no similar placebo-controlled trials have been performed. We report the results of a randomized, double-blind, placebo-controlled, dose-escalating clinical trial evaluating the safety and efficacy of allogeneic bone marrow−derived MSCs in addition to surgical treatment of refractory perianal fistulizing CD.

## Methods

### Patient Selection and Study Design

Eligible patients were men and women of at least 18 years of age with actively draining perianal fistulizing CD refractory to conventional therapies, meaning that at some time during the perianal fistula disease course, the patient must have received anti-TNF agents and, in addition, antibiotics, steroids, thiopurines, methotrexate, surgery, or combinations thereof that did not result in an adequate treatment response. Eligible patients had to have 1−2 internal openings and 1−3 fistula tracts. Additional criteria for inclusion were diagnosis of CD at least 3 months before enrollment, CD Activity Index (CDAI) score of $<250$ at screening and baseline, stable dose of current drugs (mesalamine and steroids $\geq$4 weeks; immunosuppressive drugs $\geq$8 weeks; anti-TNF agents $\geq$8 weeks), which were continued during the entire study period. Patients were not allowed to use antibiotics after inclusion in the trial. In addition, patients were not eligible if there was need for immediate surgery (obstruction, strictures, or abscesses); pregnancy; breastfeeding; or when they did not use adequate contraception. Additional exclusion criteria were evidence of any infection needing antibiotic treatment; rectovaginal fistulas; complex perianal fistulas with $>2$ internal openings; evidence of acute perianal infection; and presence of an anal or rectal stricture hindering the surgeon to adequately perform the intervention; active luminal disease; renal or hepatic failure; use of any investigational drug within 1 month before screening or within 5 half-lives of the investigational agent (whichever is longer); not able or willing to undergo magnetic resonance imaging (MRI); change in concomitant medication; documented human immunodeficiency virus infection; active hepatitis B, C, or tuberculosis; an opportunistic infection within 6 months before screening or a serious infection in the previous 3 months; malignancy within the past 5 years; or a history of lymphoproliferative disease.

Screening assessment included physical examination, laboratory measurements (including hepatitis B, C, human immunodeficiency virus serology, polymerase chain reaction−cytomegalovirus and polymerase chain reaction−Epstein-Barr virus, interferon gamma release assay, and $\beta$−human chorionic gonadotropin), stool cultures (including *Clostridium difficile* toxin A and B), and a chest x-ray to rule out the presence of tuberculosis. MRI was performed at screening to evaluate the exact anatomy and internal opening(s) of the fistula(s), their relation to the sphincters, and the presence of abscesses. When MRI results were nonconclusive, an examination under anesthesia was performed to determine the exact location of the fistula(s). Abscesses $>2$ cm were surgically drained at examination under anesthesia. Subsequently, the patient was re-screened before proceeding to inclusion or exclusion. At baseline, sigmoidoscopy was performed to rule out luminal inflammation. Perianal fistulas were classified using the "simple or complex" classification.[21]

All patients who did not have setons in situ after inclusion in the study received temporary setons to ensure that the internal opening was still open at time of surgical intervention. These setons were removed during the surgical intervention.

The study overview is shown in Supplementary Figure 1. Patients were enrolled in a double-blind, randomized 5:2 fashion to receive locally either $1 \times 10^7$ (group 1), $3 \times 10^7$ (group 2), or $9 \times 10^7$ (group 3) MSCs or 0.9% NaCl/5% human albumin solution with no cells (placebo group). Randomization was performed at the Immunohematology and Blood Transfusion Department by a researcher who did not have any contact with or any knowledge about the included patients. The study was posted on ClinicalTrials.gov under number NCT01144962. Dose escalation took place after all subjects in the previous group were treated and the Data Safety Monitoring Board reviewed the safety outcomes and approved study continuation. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center (LUMC) and the Central Committee on Research involving Human Subjects (The Hague, Netherlands) and all patients gave written informed consent. All data were collected in the LUMC.

### Preparation of Mesenchymal Stromal Cells

MSCs were prepared from 5 different donors from 50−100 mL bone marrow aspirates of healthy donors without a history of cancer or hereditary diseases and after written informed consent. Cells from different donors were processed separately and MSC yield from 1 donor was sufficient to create batches of $1 \times 10^7$, $3 \times 10^7$, and $9 \times 10^7$ MSCs. Therefore, MSCs from 1 donor were used to treat 1 patient in each MSC group. Before harvesting, donors underwent a medical screening, including routine serology testing for hepatitis B, C, human immunodeficiency virus, syphilis, and human T-lymphotropic virus. Upon medical indication, tuberculosis, Chagas disease, and West Nile virus infection were ruled out. Bone marrow was collected by aspiration from the iliac crest under local anesthesia and mononuclear cells were subsequently isolated by Ficoll separation techniques. Cells were then washed and resuspended in MSC culture medium (Dulbecco's modified Eagle's medium−low glucose/penicillin/streptomycin/10% fetal calf serum), plated in tissue culture flasks and incubated at 37°C and 5% $CO_2$. MSCs were expanded according to the standardized LUMC protocol for expansion of MSCs. Twice a week, cultures were

microscopically examined and medium was refreshed. Cells were trypsinized when >70% confluence was reached and MSC half products (passage 1) of various sizes were cryopreserved with 10% dimethyl sulfoxide. The half products were subsequently thawed for further expansion, enabling the use of MSC products with similar passage numbers from all 5 donors in each study group.

Two weeks before the intervention was planned, the patient was randomized to receive either MSCs or placebo (Supplementary Figure 1). When the patient was randomized to receive MSCs, cryopreserved MSC half product was thawed, washed, and replated in MSC culture medium for expansion to sufficient number of cells (maximally 2 passages). MSCs were then harvested and suspended at study group−dependent final concentrations in 5 mL 0.9% NaCl/5% human albumin solution divided over 2 syringes with 2.5 mL cell suspension each. This end product was released when it met the following criteria: >90% of the cells $CD73^+/CD90^+/CD105^+$, ≤1% of the cells $CD45^+$, ≤0.01% of the cells $CD3^+$, no microbial contamination (visual screening and BacTec culture) at 3−4 days before harvest, and a final cell product with spindle-shaped cell morphology and a colorless cell suspension appearance devoid of cell aggregates. Viability was determined by Trypan blue exclusion staining in a Bürker chamber. Study group−dependent final concentration of the final product was based on the number of viable cells. Placebo consisted of 2× 2.5 mL 0.9% NaCl/5% human albumin solution.

## Surgical Intervention

Surgery was performed under general anaesthesia by surgeons with expertise in inflammatory bowel disease surgery. Two surgeons performed the procedures. MSC or placebo implantation was preceded by surgical localization of the internal opening, removal of seton(s), curettage of the fistulous tract(s), trimming of the mucosa and skin of the internal and external opening, respectively, and closure of the internal opening with a polydioxanone II 4/0 suture to diminish fecal contamination of the fistula tract. Subsequently, the 5 mL of MSC or placebo suspension was divided in the following 2 steps: the first syringe with 2.5 mL MSCs or placebo was injected via the anus in the wall at 4 quadrants and equal volume around the closed internal opening. In case of 2 internal openings, the MSCs or placebo were divided over both closed internal openings in equal volumes. The second volume was injected in the wall as close as possible to the internal opening by introducing the syringe as far as possible into the fistula tract via the external opening. In case of more external openings, the MSCs or placebo were divided over all fistulas in equal volumes.

## Assessments

**Safety.** Follow-up visits after surgical intervention took place at weeks 6, 12, and 24 (Supplementary Figure 1). Safety was assessed blindly by a physician by monitoring for (serious) adverse events and changes in vital signs at time of surgical intervention with MSC or placebo injection at the day of treatment and at all follow-up visits. Routine laboratory measurements were performed and complications after surgery (eg, bleeding, wound infection, and perianal abscesses) were assessed blindly at weeks 6, 12, and 24 by a surgeon other than the surgeon who performed the surgical intervention with MSC or placebo injection. The perianal area was examined every study visit to assess possible detrimental effects of local injections, such as abnormal tissue formation. The rectum was also evaluated at week 12 during sigmoidoscopy. The primary safety end point was the incidence of (serious) adverse events at week 12. Toxicity grade of adverse events was determined using the Common Terminology Criteria for Adverse Events (version 3.0). Secondary end points included the incidence of surgical intervention and infections at week 12 and 24.

**Efficacy.** To evaluate fistula healing, a physician assessed blindly fistula discharge by gentle finger pressure at the external opening. In addition, patients underwent MRI at week 12. Fistula tracts were classified blindly by a radiologist and then compared with MRI at screening. At baseline, week 12 and 24 digital photographs were taken of the perianal area. The primary efficacy end point at week 12 was defined as a reduction in the number of draining fistulas determined by absence of discharge at physical examination and absence of collections of ≥2 cm directly related to the treated fistula tracts as measured by MRI. Secondary end points included changes in Perianal Disease Activity Index (PDAI), adapted Vaizey fecal incontinence score, CDAI, endoscopic scores (CD Endoscopic Index of Severity and Simplified Endoscopic Activity Score for CD), quality of life using the Short Inflammatory Bowel Disease Questionnaire and Short Form (SF)-36 score, and C-reactive protein from baseline to weeks 12 and 24.

## Laboratory Methods for Supportive Research

**Cytokine Measurements.** Homogenates were prepared from rectal biopsies taken at endoscopy at baseline and week 12 (n = 21), and curettage material obtained at surgical intervention (n = 20) with a Potter-Elvehjem glass homogenizer at 4°C in Greenberger lysis buffer (150 mM NaCl, 15 mM Tris [pH 7.4], 1 mM $MgCl_2$, and 1% Triton X-100). Samples were centrifuged for 15 minutes (11,000*g* at 4°C) and stored at −80°C. The BCA Protein Assay Kit (Thermo Scientific Pierce, Etten-Leur, The Netherlands) was used to determine the total concentration of protein in the samples and cytokine levels of interleukin (IL)- 8, IL-1$\beta$, IL-6, IL-10, TNF and IL-12p70 were measured using the Cytometric Bead Array System (BD Biosciences, San Diego, CA) following manufacturer's instructions. Data were analyzed with FlowJo software (version 8.7.1.; TreeStar Inc, Ashland, OR). Cytokine levels measured were corrected for the amount of total protein in the homogenate.

## Statistical Analysis

In this early phase 2 study, a sample size calculation was not performed. To compare 2 groups with numerical values, parametric or nonparametric analyses were performed using an unpaired Student *t* test or Mann-Whitney U test, respectively. Paired data were compared using the unpaired *t* test. Categorical data were analyzed with Fisher's exact test. Data were analyzed using SPSS statistical software package (2011, version 20.0, IBM SPSS Statistics for Windows, Version 20.0., IBM Corp, Armonk, NY) or GraphPad Prism software (version 5.01, San Diego, CA) and expressed as means ± SEM. $P \leq .05$ was considered statistically significant.

All authors had access to the study data and have reviewed and approved the final manuscript.

## Results

### Study Population

A total of 80 patients from all over the Netherlands were referred to the LUMC to evaluate their eligibility for this clinical trial. Of these 80 patients, 47 patients (58.8%) did not meet the inclusion criteria and 33 patients underwent screening, of which 12 patients did not meet the inclusion criteria on MRI or examination under anesthesia (Supplementary Material). Finally, 21 eligible patients were randomized, 12 were male (57.1%) and the mean age was 38.0 years (range, 21−54 years). The first patient received intervention in June 2012, the last patient in July 2014. Additional baseline characteristics of the patients are summarized in Table 1.

Mean fistula duration was 5.5 years (range, 1−19 years), most were complex fistulas with a trans-sphincteric route (both 65.2%) and the internal opening was predominantly observed at 6 o'clock (52.2%). The external openings were mostly located at 5, 6, and 7 o'clock (all 18.8%). A detailed overview of perianal fistula characteristics at baseline is shown in Table 2. The primary end points were met in all patients. For the secondary end points, 1 patient in group 2 was not able to undergo week-12 endoscopy and 1 patient in group 2 did not complete the week-24 questionnaires.



**Table 1.** Baseline Characteristics of the 21 Included Patients

| | Group 1 (n = 5) | Group 2 (n = 5) | Group 3 (n = 5) | Placebo (n = 6) |
|---|---|---|---|---|
| Age, *y*, at inclusion | | | | |
| Mean (SEM) | 40.4 (4.6) | 40.8 (1.7) | 33.4 (5.2) | 37.3 (3.6) |
| Min−max | 27−54 | 37−47 | 21−48 | 27−49 |
| Male, n (%) | 4 (80.0) | 4 (80.0) | 1 (20.0) | 3 (50.0) |
| Smoker, n (%) | | | | |
| Yes | 1 (20.0) | 1 (20.0) | 1 (20.0) | 2 (33.3) |
| No | 2 (40.0) | 3 (60.0) | 3 (60.0) | 1 (16.7) |
| Past | 2 (40.0) | 1 (20.0) | 1 (20.0) | 3 (50.0) |
| Duration of CD, *y* | | | | |
| Mean (SEM) | 7.6 (1.1) | 16.8 (4.0) | 13.2 (4.1) | 6.8 (2.9) |
| Min−max | 5−11 | 5−28 | 2−23 | 1−20 |
| Montreal classification CD, n (%) | | | | |
| L1 | 1 (20.0) | 1 (20.0) | 2 (40.0) | 1 (16.7) |
| L2 | 3 (60.0) | 2 (40.0) | 1 (20.0) | 2 (33.3) |
| L3 | 1 (20.0) | 2 (40.0) | 2 (40.0) | 2 (33.3) |
| L3+L4 | 0 | 0 | 0 | 1 (16.7) |
| Stoma[a] | 0 | 1 (20.0) | 1 (20.0) | 2 (33.3) |
| CDAI at baseline | | | | |
| Mean (SEM)[a] | 80.2 (12.1) | 203.3 (51.2) | 57.3 (14.1) | 75.8 (28.2) |
| Min−max | 44−115 | 59−283 | 38−99 | 20−148 |
| Medication, n (%) | | | | |
| Current | | | | |
| Mesalamine | 1 (20.0) | 2 (40.0) | 0 | 0 |
| 6-MP | 1 (20.0) | 1 (20.0) | 2 (40.0) | 0 |
| ADA | 3 (60.0) | 2 (40.0) | 4 (80.0) | 4 (66.7) |
| AZA | 1 (20.0) | 2 (40.0) | 1 (20.0) | 2 (33.3) |
| CS | 1 (20.0) | 0 | 0 | 0 |
| IFX | 2 (40.0) | 1 (20.0) | 1 (20.0) | 2 (33.3) |
| MTX | 1 (20.0) | 1 (20.0) | 0 | 1 (16.7) |
| Previous | | | | |
| Mesalamine | 2 (40.0) | 2 (40.0) | 2 (40.0) | 2 (33.3) |
| 6-MP | 1 (20.0) | 1 (20.0) | 1 (20.0) | 1 (16.7) |
| AB | 3 (60.0) | 3 (60.0) | 3 (60.0) | 5 (83.3) |
| ADA | 0 | 3 (60.0) | 1 (20.0) | 0 |
| AZA | 4 (80.0) | 2 (40.0) | 3 (60.0) | 2 (33.3) |
| CER | 0 | 1 (20.0) | 0 | 0 |
| CS | 5 (100) | 4 (80.0) | 5 (100) | 4 (66.7) |
| IFX | 3 (60.0) | 3 (60.0) | 2 (40.0) | 2 (33.3) |
| MTX | 1 (20.0) | 1 (20.0) | 0 | 0 |
| TGN | 0 | 1 (20.0) | 1 (20.0) | 0 |

AB, antibiotics; ADA, adalimumab; AZA, azathioprine; CER, certolizumab; CS, corticosteroids; IFX, infliximab; 6-MP, 6-mercaptopurine; MTX, methotrexate; TGN, thioguanine.
[a]CDAI was not calculated in patients with a stoma.

**Table 2.** Characteristics of the Draining Perianal Fistulas in the Included Patients

| Characteristics | Group 1 (n = 5) | Group 2 (n = 5) | Group 3 (n = 5) | Placebo (n = 6) |
|---|---|---|---|---|
| Duration of fistulas, *y* | | | | |
| Mean (SEM) | 3.6 (0.7) | 5.4 (2.5) | 9 (3.2) | 4.2 (1.1) |
| Min–max | 2–5 | 1–13 | 2–19 | 1–8 |
| PDAI at baseline | | | | |
| Mean (SEM) | 4.4 (0.5) | 3.8 (0.8) | 5 (1.1) | 5.2 (0.9) |
| Min–max | 3–6 | 2–6 | 3–9 | 3–8 |
| Horseshoeing, n (%) | | | | |
| Intralevator | 1 (20.0) | 2 (40.0) | 1 (20.0) | 4 (66.7) |
| Intersphincteric | 1 (100) | 1 (50.0) | 0 | 2 (50.0) |
| Abscess, n (%) | | | | |
| Superficial | 0 | 1 (50.0) | 1 (100) | 2 (50.0) |
| Supralevator | 3 (60.0) | 1 (20.0) | 2 (40.0) | 2 (33.3) |
| Internal openings | 3 (100) | 0 | 2 (100) | 2 (100) |
| Internal openings, n (%) | 0 | 1 (100) | 0 | 0 |
| 1 | 5 (100) | 5 (100) | 5 (100) | 8 (100) |
| 2 | 5 (100) | 5 (100) | 5 (100) | 4 (66.7) |
| Internal opening with respect to rectum, n (%) | 0 | 0 | 0 | 2 (33.3) |
| Below | 4 (80.0) | 2 (40.0) | 2 (40.0) | 5 (62.5) |
| At | 1 (20.0) | 3 (60.0) | 3 (60.0) | 3 (37.5) |
| Route of fistula, n (%) | | | | |
| Intersphincteric | 1 (20.0) | 1 (20.0) | 0 | 1 (12.5) |
| Trans-sphincteric | 3 (60.0) | 2 (40.0) | 5 (100) | 5 (62.5) |
| Suprasphincteric | 1 (20.0) | 0 | 0 | 0 |
| Extrasphincteric | 0 | 2 (40.0) | 0 | 1 (12.5) |
| Superficial | 0 | 0 | 0 | 1 (12.5) |
| External openings | | | | |
| External openings, n (%) | 9 (100) | 7 (100) | 7 (100) | 9 (100) |
| 1 | 2 (40.0) | 3 (60.0) | 3 (60.0) | 4 (66.7) |
| 2 | 2 (40.0) | 2 (40.0) | 2 (40.0) | 1 (16.7) |
| 3 | 1 (20.0) | 0 | 0 | 1 (16.7) |
| Classification fistula, n (%)[21] | | | | |
| Simple | 2 (40.0) | 1 (20.0) | 1 (20.0) | 2 (33.3) |
| Complex | 3 (60.0) | 4 (80.0) | 4 (80.0) | 4 (66.7) |

## *Efficacy*

Six weeks after treatment, all draining fistulas had healed in 3 of 5 (60.0%), 4 of 5 (80.0%), and 1 of 5 (20.0%) of the patients in groups 1, 2, and 3, respectively, and in 1 of 6 (16.7%) of the patients who received placebo, as determined by absence of discharge at physical examination and absence of ≥2 cm collections on MRI ($P = .08$ group 2 vs placebo). At week 12, all draining perianal fistulas were healed in 2 of 5 (40.0%), 4 of 5 (80.0%), and 1 of 5 (20.0%) patients in groups 1, 2, and 3 vs 2 of 6 (33.3%) of placebo-treated patients (Figure 1*A*; group 1, 2, and 3 vs placebo; all NS). Fistula-healing rates persisted throughout week 24 in all 3 MSC groups. In group 1, fistula healing increased to 4 of 5 (80.0%) at week 24. Analysis of all individual fistulas at week 6 demonstrated complete healing in 4 of 9 (44.4%), 6 of 7 (85.7%), and 2 of 7 (28.6) in groups 1, 2, and 3, respectively, vs 2 of 9 (22.2%) after treatment with placebo ($P = .04$ group 2 vs placebo). At week 12 after treatment,

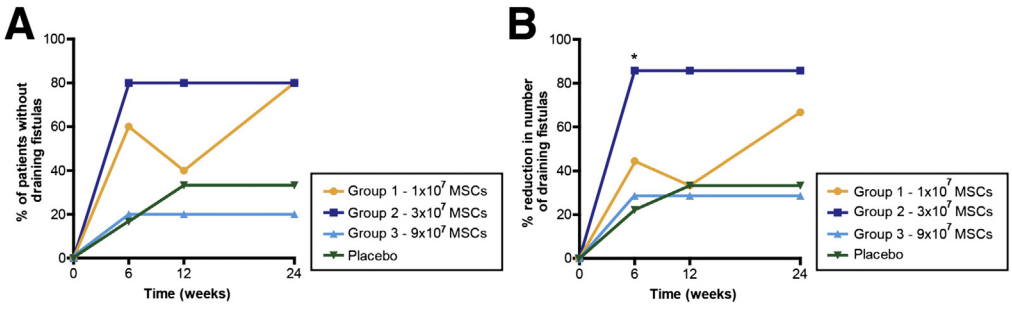


**Figure 1.** Efficacy outcomes. (*A*) Percentage of patients per group without draining perianal fistulas at weeks 6, 12, and 24. (*B*) Percentage of reduction in the number of draining perianal fistulas per group at weeks 6, 12, and 24. At week 6, the percentage of reduction in the number of draining perianal fistulas was significantly higher in patients treated with $3 \times 10^7$ MSCs (group 2) compared with patients in the placebo group ($P = .04$).

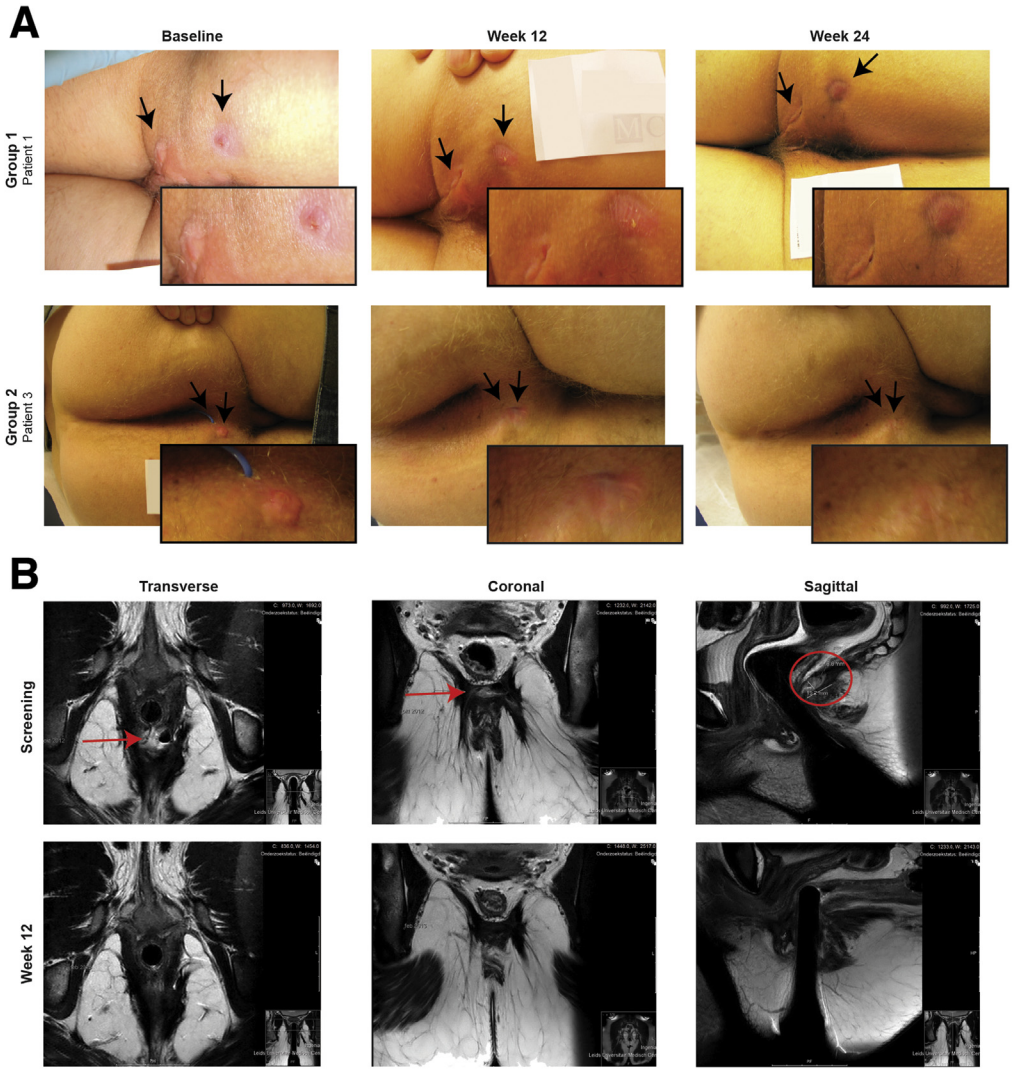


**Figure 2.** Efficacy images. (*A*) Representative digital photos at baseline, weeks 12 and 24 of the perianal area of 2 patients after local treatment with $1 \times 10^7$ MSCs (group 1 patient 1) or $3 \times 10^7$ MSCs (group 2 patient 3). In the *frames*, a zoom-in of the external openings. (*B*) Transverse, coronal, and sagittal T2-weighted magnetic resonance image of the perianal area at baseline and week 12. Patient 4 in group 1 had 1 trans-sphincteric fistula with connection to the lumen at 6 o'clock with a seton in situ (*transverse image*). A superficial abscess within the inclusion criteria was observed at the sagittal image. Twelve weeks after the fistula was treated with $1 \times 10^7$ MSCs no fistula or abscess was observed at MRI.

complete fistula healing was observed in 3 of 9 (33.3%), 6 of 7 (85.7%), and 2 of 7 (28.6%) in group 1, 2 and 3 vs 3 of 9 (33.3%) in placebo ($P = .06$ group 2 vs placebo) (Figure 1*B* and illustrated in Figure 2*A*). At week 24, healing was observed in 6 of 9 (66.7%), 6 of 7 (85.7%), and 2 of 7 (28.6%) in groups 1, 2, and 3, respectively, vs 3 of 9 (33.3%) in placebo ($P = .06$, group 2 vs placebo). The characteristics of fistula healing in relation to MSC donor are indicated in the Supplementary Material and Supplementary Figure 2*A* and *B*.

MRI evaluation at week 12 revealed the presence of $\geq$2 cm collections directly related to the treated fistulas tracts in 2 patients (1 in group 1 and 1 in the placebo group). In 2 other patients (1 in group 2 and 1 in group 3), an abscess was formed after week 12. No de novo fistulas were observed. At week-12 MRI, 3 patients showed no active fistulas anymore (1 in group 1 and 2 in group 2; see Figure 2*B*). Decreased amounts of fistula fluid were observed in 2 of 5 (40.0%), 2 of 5 (40.0%), and 1 of 5 (20.0%) in groups 1, 2, and 3, respectively, vs 1 of 6 (16.7%) in the placebo group. All patients with no or less fluid in the fistulas at the week-12 MRI also had a clinically reduction in the number of draining fistulas.

PDAI scores at weeks 0, 12, and 24 correlated well with therapy efficacy observed with physical examination and MRI. In group 1, PDAI scores decreased from 4.4 to 1.8 during 24 weeks. In group 2, this PDAI decrease was most prominent and significantly lower at week 12 compared with baseline, as well as with placebo treatment at week 12 ($P = .03$ group 2 from baseline to week 12; $P = .04$ week 12 group 2 vs placebo group; Figure 3). In group 3, PDAI did not change over time and was comparable with placebo treatment. Effects of MSC treatment on the secondary end points are indicated in Supplementary Tables 1 and 2.

### Safety

All patients tolerated the local injections of MSCs well, no infusion reactions during or directly after the surgical intervention occurred. One patient in group 2 developed a fever (39.7°C) 6 hours after surgery, probably related to a stenosis dilatation of the anal canal before surgery.

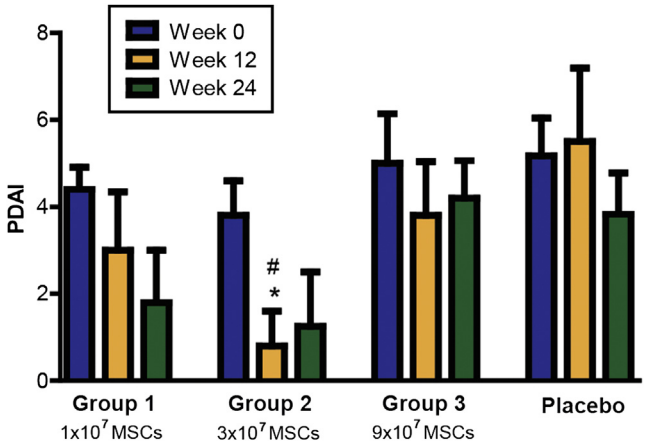


**Figure 3.** PDAI scores per study visit in all groups. Mean PDAI score at week 12 was significantly decreased compared with baseline PDAI score after treatment with $3 \times 10^7$ MSCs (group 2; $P = .03$). In addition, treatment with $3 \times 10^7$ MSCs (group 2) resulted in a significantly lower PDAI score at week 12 compared with mean PDAI score in the placebo group ($P = .04$). *Bars* represent mean and SEM.

Hospitalization was prolonged and one dose of cefuroxime/metronidazole was prescribed. The day after surgery, this patient developed abdominal pain with diarrhea and elevated C-reactive protein levels, but feces, urine, and blood cultures were negative. No abnormalities were observed on chest x-ray. No microbial contamination in the supernatant of the last washing step before packaging the MSCs for injection was found, and 2 days later the patient was discharged in good condition.

During follow-up visits, all adverse events were recorded (Table 3). All patients reported for approximately 1 week symptoms of postoperative anal pain and pus and/or blood discharge from the fistula or anus (not shown in Table 3). No changes in vital signs and no wound infections or bleedings as a result of surgical intervention were observed during the study period. In addition, no abnormal tissue formation at the perianal area by physical examination or in the rectum at endoscopy at week 12 was found. One patient in each group developed a perianal abscess that required surgical drainage at weeks 12, 16, 21, and 18 in groups 1, 2, 3, and placebo, respectively. The patients with an abscess in groups 1, 3, and placebo needed seton drainage. The fistula of the patient in group 2 was healed at week 24. The 3 patients in the placebo group with a painful perianal swelling were treated with antibiotics. None of the adverse events were judged to be related to MSC injection.

One of the patients treated with $1 \times 10^7$ MSCs developed an adenocarcinoma of the cecum with peritoneal carcinomatosis >15 months after the surgical intervention. Baseline and week-12 endoscopy of the rectum revealed no abnormalities. In addition, in retrospect, the last endoscopy of the entire colon and the biopsies taken at that time (June 2011) were completely normal. Further evaluation revealed that the uncle of this patient died from colon cancer at the age of 42.

**Table 3.** Adverse Events Reported During the Study Period of 24 Weeks After Surgical Intervention

| Adverse event | Group 1, n | Group 2, n | Group 3, n | Placebo, n |
|---|---|---|---|---|
| Blood from fistula | 1 | — | — | — |
| Perianal swelling | | | | |
| Painful | 1 | — | — | 3 |
| Not painful | 1 | — | — | 1 |
| Abscess | 1 | 1 | 1 | 1 |
| Painful anal sphincters | — | — | 1 | — |
| Fissura ani | 1 | — | — | — |
| Anal blood | — | — | — | 1 |
| Anal pus | — | 1 | — | — |
| Thrombosed hemorrhoid | — | — | — | 1 |
| Pimples | | | | |
| Buttocks | — | — | 1 | 1 |
| Abdomen | — | — | — | 1 |
| Activity CD | | | | |
| Mild | 1 | — | — | 1 |
| Exacerbation | 1 | — | — | — |
| Abdominal pain | 1 | 1 | 3 | — |
| Diarrhea | 1 | — | — | 1 |
| Flatulence | — | — | — | 1 |
| Nausea | — | — | 1 | — |
| Vomiting | — | — | 1 | — |
| Lack of appetite | 1 | — | — | — |
| Pneumonia | — | 1 | — | — |
| Common cold | 5 | 2 | 1 | 2 |
| Otitis | — | 1 | — | — |
| Headache | — | 2 | — | — |
| Back pain | — | — | 1 | — |
| Rosacea | 1 | — | — | — |
| Cold sore | 1 | — | — | — |

### *Cytokine Levels in Rectal Biopsies and Fistula Curettage Material*

In order to study MSC healing mechanisms and avoiding invasive fistula biopsies that would compromise fistula healing, we compared rectum cytokine levels (baseline and week 12) with fistula curettage obtained at surgery. Mean levels of IL-8, IL-1$\beta$, and IL-6 in the rectal biopsies were similar at baseline and after surgical intervention at week 12 (Figure 4), irrespective of treatment. No differences in the levels of these cytokines were observed at week 12 after treatment with $1 \times 10^7$, $3 \times 10^7$, or $9 \times 10^7$, or placebo (data not shown), which matched with the stable CDAI, Crohn's Disease Endoscopic Index of Severity, and Simplified Endoscopic Activity Score for CD scores during the study period. Mean levels of IL-8, IL-1$\beta$, and IL-6 were overall significantly higher in the fistula curettage material compared with mean levels in the rectal biopsies at either week 0 or 12 (all $P < .0001$). Cytokine levels of TNF, IL-10, and IL-12p70 were below the detection rate in all samples.

## Discussion

Recently, several papers have reported efficacy of local treatment of refractory perianal fistulizing CD with

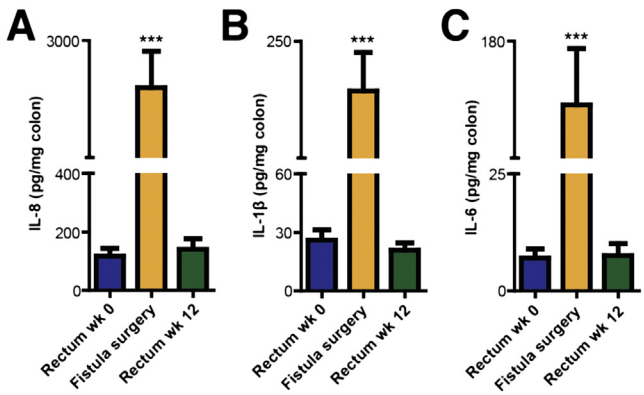


**Figure 4.** Cytokine levels in rectal biopsies and fistula curettage material. Cytokine levels were measured in homogenates of rectal biopsies and fistula curettage material. Levels of IL-8 (*A*), IL-1$\beta$ (*B*), and IL-6 (*C*) in the rectal biopsies obtained at endoscopy were comparable at baseline and week 12. Significantly higher levels of these cytokines were observed in the curettage material from the fistulas obtained at surgical intervention (all, $P < .0001$). *Bars* represent mean and SEM.

MSCs.[15–20] However, none of these trials was really placebo-controlled. Therefore, we performed an early phase 2, double-blind, placebo-controlled, randomized study addressing the use of allogeneic bone marrow–derived MSCs in the treatment of refractory perianal fistulizing CD. We showed that local administration of allogeneic bone marrow–derived MSCs was safe and feasible in patients with refractory perianal fistulizing CD.

Local treatment with lower dosages of MSCs resulted in higher fistula healing rates compared with placebo, which seems to be a dose-dependent response, as administration of $3 \times 10^7$ MSCs resulted in high fistula healing rates and $9 \times 10^7$ in rates similar to placebo treatment. Previously, the importance of MSC cell dose was described in both a sheep model of myocardial infarction and in humans with ischemic cardiomyopathy.[22,23] In both papers, a dose escalation was performed with autologous and/or allogeneic cells that were locally injected in the diseased myocardium. Only low-dose administration resulted in a beneficial effect of MSCs. The authors argued that higher cell concentrations could result in a lower survival rate and/or cell function and, secondly, that larger number of cells could behave immunogenic resulting in increased clearance or deactivation of the cells.

Fistula healing appeared to be accelerated after treatment with $3 \times 10^7$ MSCs compared with placebo treatment, as all healed fistulas in this group were already nondraining 6 weeks after the surgical intervention, as compared with at 12 weeks in patients with healed fistulas in the placebo group. Although our fistula healing rates after treatment with $3 \times 10^7$ MSCs are very encouraging, it has to be taken into account that we only included patients in whom the luminal CD was in remission. Local MSC treatment in patients with active luminal disease or more internal or external openings might be less successful and therefore merit further study. Larger phase 3 trials are warranted to confirm our observations.

Although MSC treatment was effective in groups 1 and 2, quality of life, as measured by Short Inflammatory Bowel Disease Questionnaire and Short Form-36, did not increase during the study. At baseline, PDAI scores were lower than expected for patients with perianal fistulizing CD. Both quality of life and PDAI scores consist partially of questions on restrictions in daily activities. All these questions were answered in the negative, indicating that our patients did not experience restrictions in their daily life due to the fistulas, probably because, after a mean 5.5 years, they were used to having fistulas with continuous discharge and pain, and managed to continue with their activities despite the daily discomfort. In addition, abscesses >2 cm were drained before inclusion in the trial, resulting in less anal pain, lower PDAI scores, and possibly a better quality of life.

In our study, we used allogeneic MSCs harvested from bone marrow of healthy donors. One of the advantages of allogeneic MSCs is the possibility of generating a stock with "off-the-shelf" treatment potential without the requirement to expand autologous MSCs for weeks before a patient can be treated. Immediate availability of MSCs for the treatment of fistulas is warranted, as development of abscesses requires surgery and results in a reduced quality of life. In addition, MSCs can be harvested from healthy young donors with a higher yield compared with older donors and possessing a higher regenerative and immunomodulatory potential also avoids possible disease-related effects on the autologous MSCs.[24–26] Similar to autologous MSCs, in this study, allogeneic MSCs were observed to be safe. During the study period, none of the included patients reported adverse events related to MSC injection.

One of the concerns of cell-based therapy is the safety regarding malignant transformation of the administered cells. Although no neoplasia were observed during the long-term follow-up study,[27] a recently published meta-analysis 14 papers[28] did show occurrence of malignancies, but only in patients with previous or current malignancies, no formation of de novo tumors were reported. In our study, only 1 patient (in group 1) developed an adenocarcinoma, but it was localized in the cecum. In our patient, the rectum was and remained completely normal at baseline and follow-up endoscopy of the local administration site. The local injection of MSC in this regard argues against causing cancer at a different site. Also, the patient developed this cancer >15 months after MSC therapy. Adult MSCs are reported to have a very limited lifespan in vivo; such a late cancer development seems incompatible with a direct relation to MSC administration. Lastly, the patients' uncle died from colon cancer at age 42, suggesting a hereditary component.

Although the recently published results on MSC treatment for perianal fistulizing CD are promising, there are substantial differences in the origin and preparation of MSCs and techniques of administration among these studies, making it difficult to compare.[15–20] We believe that an important step to achieving MSC efficacy is the removal of the epithelial layer present in long-standing perianal fistulas hindering fistula healing. In addition, attention must be paid to the closure of the internal opening because that is the

place where the fistula has emerged and prohibits fecal contamination of the treated fistula tract. For this reason, this was also the location of MSCs injection.

The mechanism(s) by which the administered MSCs exert their action was not studied in our trial. MSCs possess a broad range of immunomodulatory properties interfering in both the innate and adaptive immune system.[29,30] Pre- and post-MSC administration, biopsies with subsequent analysis of infiltrates could shed light whether the immunosuppressive effects of MSCs in vitro can be confirmed. It has been reported that the percentage of regulatory T cells was increased in both rectal biopsies and peripheral blood 12 months after intrafistular MSC treatment compared with baseline, suggesting a systemic effect of injected MSCs.[18] In our patients, active CD was only localized in the fistula with luminal disease in remission as reflected by the stable CDAI, Crohn's Disease Endoscopic Index of Severity, and Simplified Endoscopic Activity Score for CD during the entire study period. This was also reflected by the stable and low levels of inflammatory cytokines in the biopsies. Interestingly, examining the mean levels of proinflammatory cytokines, IL-8, IL-1$\beta$, and IL-6, in the fistula curettage material obtained at surgical intervention revealed baseline fistula cytokine levels were at least significantly higher, as found in rectal biopsies in either week 0 or 12. Unfortunately, no follow-up material of the fistulas could be studied.

In conclusion, local administration of allogeneic bone marrow−derived MSCs was found to be safe and feasible in patients with refractory perianal fistulizing CD. Local treatment with $3 \times 10^7$ MSCs showed superior fistula healing compared with placebo, and lower MSC dose seemed superior to the use of a higher dose.

## Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2015.06.014.

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The authors disclose no conflicts.

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# Supplementary Material

## *Patient Exclusion and Inclusion in More Detail*

Forty-seven patients (58.8%) did not meet the inclusion criteria and were therefore not screened because of the following reasons: patient did not want to be included in the study after oral and written information (n = 13), nondraining fistula (n = 8), presence of a pouch (n = 6) or a rectovaginal fistula (n = 3), fistula not proven refractory (n = 4), malignancy in the previous 5 years (n = 4), no internal opening or >2 internal openings present (both n = 3), no rectum in situ (n = 1), heart failure (n = 1), or not willing to receive treatment for latent tuberculosis (n = 1).

Twelve patients did not meet the inclusion criteria on MRI or examination under anesthesia (EUA) at screening because of the lack of an internal opening on MRI or EUA (both n = 3), >2 internal openings at EUA (n = 1), no external opening at baseline (n = 1), or the presence of a rectovaginal fistula at EUA or during the surgical intervention (n = 3 and n = 1, respectively). The latter patient was replaced.

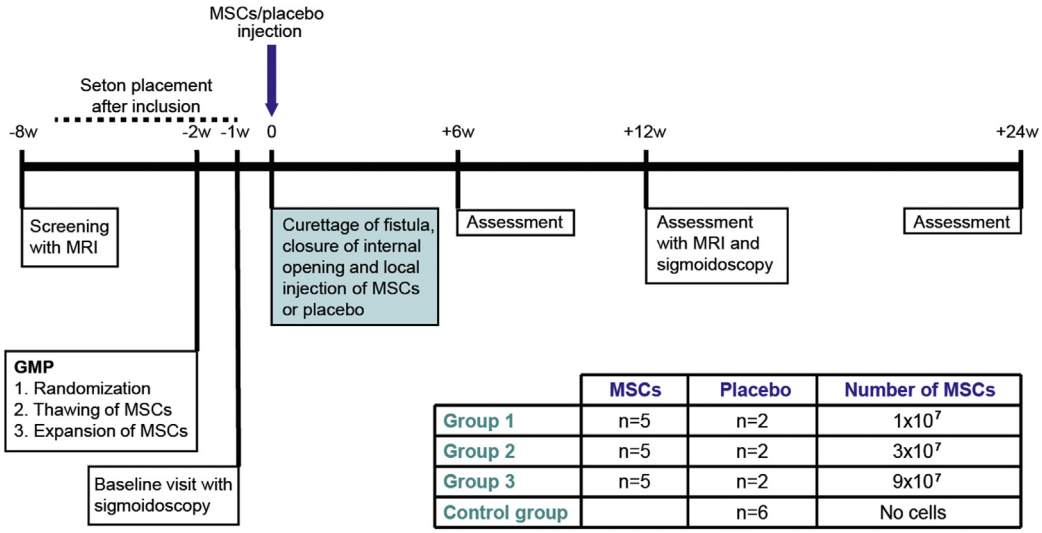
Two patients in group 2 had a CDAI score >250 at baseline. Both patients were included because 1 patient was incontinent at baseline, resulting in fecal soiling approximately 20 times a day, increasing the CDAI score without active luminal disease. The second patient had an exacerbation of CD at the ileocolonic anastomosis in the month before baseline visit, but no active disease was observed during endoscopy of the rectum and sigmoid.

More details regarding fistulas: at surgical intervention, some patients still had an abscess related to the fistula tract, however, those were always <2 cm. Abscesses were drained during surgical intervention before injection of MSCs or placebo. In the placebo group, 2 patients had a fistula with 1 external opening that horseshoed resulting in 2 internal openings.

MSCs from 5 healthy donors were used in this trial (Supplementary Figure 1*A* and *B*). The number of MSCs harvested from 1 donor was sufficient to treat 1 patient in each group. Efficacy of MSCs from donor A, C, D, and E at week 24 was high. Five fistulas were treated with MSCs from donor A and of these 5 fistulas 4 were nondraining at week 24 (80%). Fistulas treated with MSCs from donor C, D, or E were nondraining in 75.0% (3 of 4 fistulas of all 3 donors) at week 24. However, fistula healing rate after treatment with donor B was 1 of 6 (16.7%). This might be explained by the fact that the only 2 patients with 3 fistulas received MSCs from donor B or placebo. In addition, injection of MSCs from donor B in patient 2 of group 3 was found to be performed >4 hours after the MSCs were prepared due to an emergency surgery that needed to be scheduled before this patient. This was also the case in patient 5 of group 2, who received MSCs from donor C.

All patients received MSCs passage 2, except for 1 patient in group 2 who received MSCs passage 3. This patient replaced the patient who was excluded during surgical intervention because of a rectovaginal fistula. We were able to treat this patient with MSCs of the same donor as the patient who was excluded, however, 1 passage extra was needed to generate sufficient number of MSCs.

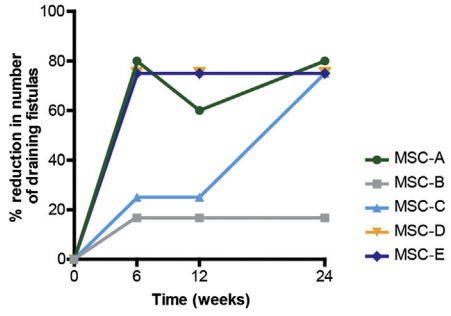
Although the sample size per group was small, making it impossible to perform statistical tests within each group, we analyzed the difference in the number of patients with and without draining fistulas at week 12 and 24 based on fistula route (Supplementary Table 3), the simple/complex classification (Supplementary Table 4), the location of the internal opening (Supplementary Table 5), and the number of draining fistulas at baseline (Supplementary Table 6) in the entire group (including placebo) with the Fisher's exact test in case of 2 groups with categorical data and the Pearson's $\chi^2$ in case of >2 groups with categorical data. At week 12 and 24 no statistically significant differences were observed in the number of patients without draining fistulas and fistula route, the simple/complex classification, the location of the internal opening, or the number of draining fistulas at baseline. In the subanalysis comparing the closure rate of patients with an extrasphincteric fistula to patients with 1 of the other 4 routes of fistulas, we did observe a statistically significant difference between the number of patients without draining fistulas at week 12 and the presence of an extrasphincteric fistula at baseline ($P = .047$). However, this difference is not significant anymore at week 24, as the only extrasphincteric fistula in the placebo group recurred. The extrasphincteric fistulas of the other 2 patients that were both treated with $3 \times 10^7$ MSCs were still healed at week 24. Although we observed a statistically significant difference at week 12, the number of patients with a draining extrasphincteric fistula at baseline was too low (n = 3).




| | MSCs | Placebo | Number of MSCs |
|---|---|---|---|
| Group 1 | n=5 | n=2 | $1x10^7$ |
| Group 2 | n=5 | n=2 | $3x10^7$ |
| Group 3 | n=5 | n=2 | $9x10^7$ |
| Control group | | n=6 | No cells |

**Supplementary Figure 1.** Study overview.

**A.**




**B.**

| Group | Patient (nr) | Draining fistula(s) at baseline (n) | Donor (nr) | Reduction of draining fistulas Week 6 | Week 12 | Week 24 |
|---|---|---|---|---|---|---|
| 1 | 1 | 2 | MSC-A | 2/2 | 1/2 | 2/2 |
| | 2 | 3 | MSC-B | 0/3 | 0/3 | 0/3 |
| | 3 | 2 | MSC-C | 0/2 | 0/2 | 2/2 |
| | 4 | 1 | MSC-D | 1/1 | 1/1 | 1/1 |
| | 5 | 1 | MSC-E | 1/1 | 1/1 | 1/1 |
| *Total* | | *9* | | *4/9* | *3/9* | *6/9* |
| 2 | 1 | 1 | MSC-B | 1/1 | 1/1 | 1/1 |
| | 2 | 2 | MSC-E | 2/2 | 2/2 | 2/2 |
| | 3 | 2 | MSC-D | 2/2 | 2/2 | 2/2 |
| | 4 | 1 | MSC-A | 1/1 | 1/1 | 1/1 |
| | 5 | 1 | MSC-C | 0/1 | 0/1 | 0/1 |
| *Total* | | *7* | | *6/7* | *6/7* | *6/7* |
| 3 | 1 | 1 | MSC-E | 0/1 | 0/1 | 0/1 |
| | 2 | 2 | MSC-B | 0/2 | 0/2 | 0/2 |
| | 3 | 2 | MSC-A | 1/2 | 1/2 | 1/2 |
| | 4 | 1 | MSC-C | 1/1 | 1/1 | 1/1 |
| | 5 | 1 | MSC-D | 0/1 | 0/1 | 0/1 |
| *Total* | | *7* | | *2/7* | *2/7* | *2/7* |
| Placebo | 1 | 1 | n/a | 0/1 | 0/1 | 0/1 |
| | 2 | 2 | n/a | 2/2 | 2/2 | 1/2 |
| | 3 | 1 | n/a | 0/1 | 0/1 | 0/1 |
| | 4 | 3 | n/a | 0/3 | 0/3 | 0/3 |
| | 5 | 1 | n/a | 0/1 | 0/1 | 1/1 |
| | 6 | 1 | n/a | 0/1 | 1/1 | 1/1 |
| | | *9* | | *2/9* | *3/9* | *3/9* |

**Supplementary Figure 2.** Fistula healing in relation to MSC donor. (*A*) Percentage of reduction in the number of draining perianal fistulas per MSC donor at weeks 6, 12, and 24. (*B*) Overview of efficacy after treatment with MSCs or placebo.

**Supplementary Table 1.** Effects of Mesenchymal Stromal Cells on the Secondary End Points

| | Group 1 (n = 5) | Group 2 (n = 5) | Group 3 (n = 5) | Placebo (n = 6) |
|---|---|---|---|---|
| CDAI[a] | | | | |
| Week 0 | 80.2 (12.1) | 203.3 (51.2) | 57.3 (14.1) | 75.8 (28.2) |
| Week 12 | 76.6 (26.1) | 154.8 (34.0) | 74.3 (15.7) | 130.3 (45.0) |
| Week 24[b] | 64.8 (13.5) | 171.3 (16.7) | 80.8 (25.1) | 58.0 (21.9) |
| CDEIS | | | | |
| Week 0 | 0 | 4.6 (4.6) | 0.6 (0.6) | 9.1 (5.8) |
| Week 12 | 1.2 (1.2) | 0[c] | 0.6 (0.6) | 6.5 (4.8) |
| SES-CD | | | | |
| Week 0 | 0 | 1.0 (1.0) | 0.2 (0.2) | 2.0 (1.3) |
| Week 12 | 0.6 (0.6) | 0[c] | 0.2 (0.2) | 1.8 (1.6) |
| Adapted Vaizey incontinence | | | | |
| Week 0 | 1.8 (0.6) | 3.7 (1.9) | 4.0 (1.1) | 2.3 (0.3) |
| Week 12 | 0.6 (0.4) | 1.0 (1.0) | 3.3 (0.9) | 2.0 (0.8) |
| Week 24[b] | 0.8 (0.8) | 1.0 (1.0) | 3.0 (1.0) | 1.0 (0.6) |
| CRP | | | | |
| Week 0 | 4.4 (2.1) | 10.8 (7.1) | 0 | 3.5 (1.7) |
| Week 12 | 5.6 (2.7) | 5.2 (4.5) | 1.2 (1.2) | 8.9 (5.4) |
| Week 24[b] | 5.7 (1.8) | 7.9 (6.8) | 0 | 4.3 (1.6) |
| sIBDQ | | | | |
| Week 0 | 61.0 (2.5) | 48.8 (3.7) | 52.8 (4.5) | 55.3 (7.0) |
| Week 12 | 57.8 (4.7) | 48.5 (3.8) | 51.5 (4.7) | 46.5 (7.2) |
| Week 24[b] | 60.0 (2.7) | 51.7 (1.7) | 50.5 (3.4) | 59.3 (3.8) |

NOTE. Values are mean (SEM).
CDEIS, Crohn's Disease Endoscopic Index of Severity; CRP, C-reactive protein; SES-CD, Simplified Endoscopic Activity Score for Crohn's Disease; sIBDQ, Short Inflammatory Bowel Disease Questionnaire.
[a]CDAI was not calculated in patients with a stoma.
[b]At week 24, one patient in group 2 did not fill in the questionnaires and blood was not drawn in this patient.
[c]One patient in group 2 was not able to undergo endoscopy at week 12, therefore, CDEIS and SES-CD were not calculated.

**Supplementary Table 2.** Short Form-36 Scores per Group at Baseline, Week 12, and Week 24

| | Group 1 (n = 5) | Group 2 (n = 5) | Group 3 (n = 5) | Placebo (n = 6) |
|---|---|---|---|---|
| Physical functioning | | | | |
| Week 0 | 96.0 (1.9) | 75.0 (8.1) | 92.0 (2.6) | 85.0 (3.7) |
| Week 12 | 92.0 (4.6) | 81.0 (10.8) | 90.0 (4.5) | 74.2 (8.9) |
| Week 24 | 97.0 (1.2) | 87.5 (5.2) | 92.0 (3.4) | 86.7 (4.4) |
| Physical role functioning | | | | |
| Week 0 | 80.0 (8.2) | 53.8 (14.1) | 75.0 (8.1) | 69.8 (9.3) |
| Week 12 | 68.8 (12.5) | 61.3 (15.5) | 77.5 (5.8) | 56.3 (13.8) |
| Week 24 | 73.8 (9.4) | 51.6 (7.8) | 71.3 (5.1) | 70.8 (6.8) |
| Bodily pain | | | | |
| Week 0 | 91.6 (5.4) | 71.6 (16.5) | 74.6 (9.7) | 62.2 (8.3) |
| Week 12 | 82.0 (7.7) | 82.8 (5.0) | 89.2 (6.6) | 52.7 (13.8) |
| Week 24 | 81.3 (13.9) | 67.5 (3.2) | 77.8 (9.8) | 74.2 (8.4) |
| General health perceptions | | | | |
| Week 0 | 68.0 (7.3) | 33.4 (8.2) | 64.6 (7.0) | 58.2 (10.1) |
| Week 12 | 58.2 (10.6) | 32.4 (8.0) | 65.0 (4.6) | 47.3 (8.4) |
| Week 24 | 61.6 (8.5) | 30.5 (10.8) | 60.2 (4.5) | 51.8 (10.1) |
| Vitality | | | | |
| Week 0 | 73.8 (6.7) | 38.8 (7.2) | 62.5 (12.5) | 64.0 (5.2) |
| Week 12 | 60.0 (5.8) | 45.0 (12.3) | 60.0 (12.3) | 50.2 (7.7) |
| Week 24 | 70.0 (7.2) | 39.1 (6.4) | 50.0 (13.6) | 63.5 (4.4) |
| Social functioning | | | | |
| Week 0 | 90.0 (4.7) | 57.5 (13.5) | 77.5 (13.9) | 87.5 (7.9) |
| Week 12 | 82.5 (5.0) | 65.0 (14.5) | 87.5 (12.5) | 60.4 (14.9) |
| Week 24 | 82.5 (5.0) | 62.5 (13.5) | 80.0 (8.5) | 87.5 (6.5) |
| Emotional role functioning | | | | |
| Week 0 | 85.0 (6.1) | 51.7 (6.7) | 68.3 (9.6) | 80.6 (10.0) |
| Week 12 | 66.7 (11.5) | 45.0 (13.1) | 83.3 (10.9) | 61.1 (15.2) |
| Week 24 | 80.0 (5.7) | 62.5 (12.0) | 71.7 (12.0) | 79.2 (7.4) |
| Mental health | | | | |
| Week 0 | 81.0 (2.9) | 51.0 (4.8) | 70.0 (9.1) | 79.2 (8.6) |
| Week 12 | 76.3 (6.3) | 53.0 (11.0) | 70.0 (6.5) | 66.7 (9.3) |
| Week 24 | 85.0 (4.5) | 53.8 (10.5) | 66.0 (7.0) | 80.0 (5.8) |

NOTE. Values are mean (SEM). At week 24, one patient in group 2 did not fill in the Short Form-36.

**Supplementary Table 3.** Fistula Healing Based on Fistula Route (n = 23 Internal Openings)

| Route of fistula | No draining fistulas at week 12 | No draining fistulas at week 24 | *P* value week 12 | *P* value week 24 |
|---|---|---|---|---|
| Intersphincteric | 2/3 | 3/3 | .096 | .207 |
| Trans-sphincteric | 4/15 | 6/15 | | |
| Suprasphincteric | 0/1 | 1/1 | | |
| Extrasphincteric | 3/3 | 2/3 | | |
| Superficial | 0/1 | 0/1 | | |

**Supplementary Table 4.** Fistula Healing Based on the Simple/Complex Classification (n = 21 Patients)

| Classification | No draining fistulas at week 12 | No draining fistulas at week 24 | *P* value week 12 | *P* value week 24 |
|---|---|---|---|---|
| Simple | 4/6 | 4/6 | .331 | .635 |
| Complex | 5/15 | 7/15 | | |

**Supplementary Table 5.** Fistula Healing Based on the Location of the Internal Opening (n = 23 Internal Openings)

| Location internal opening with respect to the rectum | No draining fistulas at week 12 | No draining fistulas at week 24 | *P* value week 12 | *P* value week 24 |
|---|---|---|---|---|
| Below | 6/13 | 7/13 | .669 | 1.000 |
| At | 3/10 | 5/10 | | |

**Supplementary Table 6.** Fistula Healing Based on the Number of Draining Fistulas at Baseline (n = 21 Patients)

| No. of draining fistulas at baseline | No draining fistulas at week 12 | No draining fistulas at week 24 | *P* value week 12 | *P* value week 24 |
|---|---|---|---|---|
| 1 | 6/12 | 7/12 | 0.417 | 0.296 |
| 2 | 3/7 | 4/7 | | |
| 3 | 0/2 | 0/2 | | |