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# Autologous Adipose Tissue-Derived Stem Cells Treatment Demonstrated Favorable and Sustainable Therapeutic Effect for Crohn's Fistula

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**Key Words.** Adipose tissue-derived stem cells • Autologous stem cells • Crohn's fistula • Complete closure of fistula • Sustained response

ABSTRACT

**Fistula is a representative devastating complication in Crohn's patients due to refractory to conventional therapy and high recurrence. In our phase I clinical trial, adipose tissue-derived stem cells (ASCs) demonstrated their safety and therapeutic potential for healing fistulae associated with Crohn's disease. This study was carried out to evaluate the efficacy and safety of ASCs in patients with Crohn's fistulae. In this phase II study, forty-three patients were treated with ASCs. The amount of ASCs was proportioned to fistula size and fistula tract was filled with ASCs in combination with fibrin glue after intralesional injection of ASCs. Patients without complete closure of fistula at 8 weeks received a second injection of ASCs containing 1.5 times more cells than the first injection. Fistula healing at week 8 after final dose injection and its sustainability for 1-year were evaluated. Healing was defined as a complete closure of external opening without any sign of drainage and inflammation. A modified per-protocol analysis showed that complete fistula healing was observed in 27/33 patients (82%) by 8 weeks after ASC injection. Of 27 patients with fistula healing, 26 patients completed additional observation study for 1-year and 23 patients (88%) sustained complete closure. There were no adverse events related to ASC administration. ASC treatment for patients with Crohn's fistulae was well tolerated, with a favorable therapeutic outcome. Furthermore, complete closure was well sustained. These results strongly suggest that autologous ASC could be a novel treatment option for the Crohn's fistula with high-risk of recurrence.** STEM CELLS *2013;31:2575–2581*

Disclosure of potential conflicts of interest is found at the end of this article.

## INTRODUCTION

Crohn's disease is an immunologically mediated inflammatory bowel disease with a reported incidence of 4.0–7.0, 7.1, and 1.34 per 100,000 persons in Europe, the U.S., and Korea, respectively [1, 2]. The etiology of Crohn's disease remains unknown. It is believed to be caused by a disfunction of mucosal T cells, ultimately leads to chronic inflammation and damage of the intestine. Uncontrolled chronic inflammation finally causes various complications in intestine such as bowel obstruction, fistulas, abscesses, and anal fissures [3].

The incidence of perianal fistula was reported in 13%–39% of patients with Crohn's disease [4, 5]. Medical treatment for Crohn's fistulae initially focused on surgical intervention accompanied by symptomatic treatment with antibiotics and immunosuppressants. Although a short-term effect is generally achieved by surgery, poor healing and a high rate of fistula recurrence are reported by many studies [6–8]. The most serious problem after surgical intervention is the relatively high incidence of postoperative anal incontinence caused by sphincter injury during the procedure [9, 10].

Conversely, available pharmacological therapies for Crohn's fistulae based on biologic agents such as infliximab do not generally reach ideal goal of treatment (e.g., complete closure of the fistula). A high recurrence rate after treatment with infliximab has also been reported, even after long-term maintenance therapy [11, 12], which suggests that infliximab

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monotherapy does not provide adequate healing. An internal pocket in the fistula tract remaining unhealed after infliximab treatment was suggested to be the predominant reason for recurrence [13, 14]. To reach a better clinical outcome, combination treatment with infliximab and surgical intervention is highly recommended for management of Crohn's fistulae [15]. Nonetheless, even this strategy does not result in a satisfactory healing for many patients.

The ideal therapeutic goal of treatment is not only complete closure of the fistula without recurrence but also preservation of anal sphincter function [12]. Unfortunately, currently available medical and/or surgical treatment is not likely to offer a cure for perianal fistulae and, as noted above, recurrence is frequently reported. Therefore, Crohn's disease patients may suffer from persistent unhealed fistulae and a poor quality of life.

Together with active research in the field of bone marrow-derived mesenchymal stem cells (BM-MSCs) and hematopoietic stem cells [16–18], autologous or allogenic adipose tissue-derived stem cells (ASCs) [14, 19-23] have been studied for management of Crohn's disease and other disorders. Of particular relevance to this study, ASCs could be considered to be safe and efficacious therapeutic tools for the treatment of Crohn's fistulae [20]. Importantly, ASCs do not cause fecal incontinence after injection into the lesion site in Crohn's disease patients with rectovaginal fistulae [19]. As such, ASCs have been granted orphan drug designation in Europe for the treatment of complex fistulae, including Crohn's fistulae. Significant healing was observed in 71% of the subjects in a clinical study of patients with complex fistulae [23].

Prior to this phase II clinical study, a phase I dose-escalation clinical study with ASCs manufactured by Anterogen Co., Ltd. (Seoul, Korea) demonstrated the safety and therapeutic potential of these cells for the treatment of Crohn's fistulae [24]. Therefore, this study was conducted to evaluate the efficacy and safety of ASCs in a statistically significant number of patients suffering from Crohn's fistulae using the ASC dose determined by the previous phase I study.

## Materials and Methods

### Patients

Eligible patients were aged >18 years and were diagnosed with perianal fistulae associated with Crohn's disease. Exclusion criteria included a medical or family history of variant Creutzfeldt–Jakobs disease; activated severe Crohn's disease; perianal fistulae >2 cm in diameter; autoimmune disease or inflammatory bowel disease other than Crohn's disease; infectious diseases including hepatitis B virus, hepatitis C virus and human immunodeficiency virus infection; active tuberculosis (including anal tuberculosis); signs of septicemia; patients treated with infliximab within 3 months prior to ASC treatment; allergies or hypersensitivity to bovine-derived materials; sensitivity to fibrin glue; and in situ surgery for malignancy (except carcinoma) during the past 5 years. Patients were also excluded if the collection of adipose tissue was technically difficult due to low levels of fat tissue. Women of childbearing age who were unwilling to use barrier contraceptive methods or women who were breastfeeding or pregnant were also excluded.

### Study Design

This open-label, phase II study was conducted at five hospitals in South Korea. The study was initiated in January 2010 and completed in August 2012. All patients provided written informed consent to the participating hospital before joining the clinical trial. Eligible patients were enrolled based on the screening data described above and were then subjected to liposuction followed by adipose tissue extraction. If the adipose tissue obtained was insufficient to prepare the required amount of ASCs, additional fat tissue collection was performed at the discretion of the investigator.

ASCs (Anterogen Co., Ltd., Seoul, Korea) were injected into the lesion site(s) in each patient at baseline (Day 1). A second injection of ASCs was performed for patients who did not show complete closure of the fistula at 8 weeks after the first injection. Patients were monitored at baseline and at 4, 6, and 8 weeks after injection for efficacy analysis. The efficacy analysis included clinical assessment of adverse events (AEs), a physical examination, and collection and analysis of blood samples for the measurement of laboratory parameters. After week 8 visit, additional 10 months follow-up was performed for the patients who agreed to this observation study to evaluate long-term efficacy and safety of ASC injection.

The study was conducted according to Good Clinical Practice guidelines and the principles set out in the Declaration of Helsinki. The study protocol and the informed consent form were approved by institutional review boards at each study site and by the Korean Food and Drug Administration.

The sample size was determined for the number of patients with complete response that was required to demonstrate a statistically significant difference of 25% between the efficacy rate of ASC treatment at week 8 and the published efficacy rate for infliximab. An assumption was made that the rate of complete response afforded by infliximab was 46% [25], whereas that afforded by ASCs was 71%. The sample size was estimated using the Z-test with 95% confidence and 80% power for comparing the efficacy of ASCs with that of infliximab. According to this assumption, a sample size of 30 patients was calculated.

### Preparation of ASCs

To prepare ASCs, subcutaneous adipose tissue was collected by liposuction or by extraction of fat tissue under anesthesia. Fat tissue (10–40 ml) was digested in phosphate buffered saline (Hyclone, Logan, UT) containing 1% bovine serum albumin and 0.025% collagenase for 80 minutes at 37°C with intermittent shaking. The stromal vascular fraction isolated from the fat tissue was plated and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and 1 ng/ml basic fibroblast growth factor to obtain the required number of ASCs for injection. ASCs were subcultured to passages 3–4. After harvesting via trypsinization, cells were suspended in DMEM and packaged into single-use vials containing $3 \times 10^7$ cells per milliliter. All manufacturing procedures were carried out according to the Good Manufacturing Practices authorized by the Korean Food and Drug Administration.

Manufactured ASCs were characterized by their expression of the stromal cell-associated markers CD (cluster of differentiation) 10, CD13, CD29, CD44, and CD90. The cells were negative for the expression of the hematopoietic stem cell-associated markers CD34 and CD45 and the bone marrow-derived stem cell-associated marker STRO-1 [26]. Furthermore, genomic stability of ASCs was evaluated by karyotyping analysis and no evidence of tumor formation was observed in tumorigenicity study carried out with immune-compromised nude mice after repeated administration of cells. For lot release testing, ASCs were assessed for cell appearance, viability, identification, purity, content, and potency. Minimum criteria for release were 80% for cell viability and

less than 1% of CD45-positive cells for purity. In addition, ASCs were screened for contamination with adventitious agents, mycoplasma, and other bacteria, fungi, and viruses. Endotoxin should be less than 3 EU/ml.

### Surgical Procedure

Before injection with ASCs, fistula tract was thoroughly curetted and irrigated under anesthesia. After internal opening was completely sutured using 2-0 vicryl, cells were evenly injected into the submucosa surrounding internal opening and fistula tract wall. Open fistula tract was filled with a mixture of ASCs and fibrin glue (Greenplast kit, Seoul, Korea or Tisseel-fibrin sealant, Wien, Austria) using dual syringe injection system.

Dose of ASCs was determined based on the fistula size. The fistula size was determined from diameter and length of fistula, which were measured using a probe before injection. Approximately $3 \times 10^7$ cells per centimeter length when the diameter of fistula was not above 1 cm were injected and two times of cells were administered when diameter of fistula was 1 cm $< d \leq 2$ cm. If a second injection of ASC was necessary, the dose contained 1.5 times number of cells used in the first injection.

### Assessments

To evaluate fistula healing, digital photographs were taken of the lesion site at weeks 4, 6, and 8 and compared with photographs taken on Day 1 (before/after ASC injection). The primary efficacy endpoint of this study was the fraction of patients with complete healing of fistula at 8 weeks after the final injection of ASCs. Complete healing was defined as complete closure of fistula tract and internal and external openings, without drainage or any sign of inflammation. Secondary efficacy endpoints were the fraction of patients with a decrease in drainage of more than 50% and the investigator's satisfaction with ASC efficacy according to a five-point grading scale. In observation study, sustaining of fistula healing was evaluated at month 4, 6, 9, and 12. Safety evaluations included analysis of systemic tolerance, AEs, serious adverse events (SAEs), and laboratory toxicity after the injection of ASCs.

### Statistical Analysis

Modified per-protocol (PP) analysis including patients who had received final dose of ASCs and completed the efficacy evaluation at week 8 visit was preferred as primary efficacy analysis in phase II study and its extension study. Patients who received other procedures or operations involving the injection site, or who were given infliximab within 3 months prior to ASC injection or during the clinical study, were excluded from modified PP analysis. An intention-to treat (ITT) analysis was also performed based on initial treatment intent. Patients discontinuing the study were included in the efficacy analysis until the time of their discontinuation. Data analysis was performed using the chi-square test. Comparisons were performed at a significance (alpha) level of 0.05. Relationship was assessed using Pearson's correlation coefficient. Analysis was undertaken using PASW version 18.

## Results

### Study Population

Fifty patients were enrolled and a total of 43 patients were injected with ASCs in this study (Fig. 1). Of these, 70% ($n = 30$) was male and 30% ($n = 13$) was female. The mean age was 26.1 ± 5.6 years. The average duration of Crohn's disease was 54.6 ± 40.1 months, and the average duration of fistula occurrence was 46.8 ± 41.6 months. Patients whose Crohn's disease and fistula were maintained more than 4 years were 53.5% and 41.9%, respectively. Mean fistula length was 4.6 ± 1.6 cm. The type of fistula was transsphincteric in 30 patients (70%), extrasphincteric in eight patients (19%), and suprasphincteric in five patients (12%). Average injection volume of ASCs was 5.5 ± 2.8 ml per lesion site, and the average number of cells for first injection and second injection was $15.8 \times 10^7$ and $19.1 \times 10^7$, respectively (Table 1).

One out of 43 patients was withdrawn from the study before the 8-week evaluation visit due to violation of exclusion criteria (i.e., the external fistula opening was more than 2 cm in diameter); hence, 42 patients completed the first 8-week efficacy evaluation. Fifteen patients did not show complete fistula closure at 8 weeks after the first dose of ASCs. Of these, four patients discontinued the study of their own accord, and one was withdrawn by the investigator. Therefore, 10 patients were injected with a second dose of ASCs. One patient was withdrawn from the study after the second ASCs injection as that patient was lost to follow-up. In total, 36 patients completed the 8-week evaluation visit after the final dose of ASCs.

Among these 36 patients, 33 were included in the modified PP analysis. The other three patients were treated with infliximab, seton placement, or penrose drain insertion during the course of study and were included in the ITT analysis. In additional follow-up study, 27 patients who showed complete closure of fistula at week 8 after ASCs injection were enrolled to assess whether complete response and safety was sustained.

### Healing

Efficacy outcome of ASCs treatment was summarized based on the treatment cycle (one or two injection) and analysis group (Table 2). In the modified PP analysis, 82% (27/33) of the patients showed complete fistula healing at 8 weeks after the final injection of ASCs (Fig. 2). Of 27 patients with complete closure of the fistula, 26 patients achieved the response from the treatment of only one injection of ASCs. The seven patients who did not show a complete response were injected with a second dose of ASCs. One of these seven patients (14%) showed complete healing of the fistula at 8 weeks after the second ASCs injection.

Overall, six patients showed incomplete closure of fistula in the modified PP analysis, even though, five patients of them achieved fistula closure more than 50% of fistula tract with a marked decrease in drainage (more than 50%). The investigators were satisfied with the ASC efficacy in 94% of the patients. To find a predictor for fistula healing, a subgroup analysis was performed according to the diameter or length of the fistula, fistula type according to Park's classification, patient gender, and duration of Crohn's disease. However, no statistical significance was found between any of these parameters and fistula healing.

Based on the results of the ITT analysis, 64.3% (27/42) of the patients showed fistula healing after the first injection of ASCs. As noted above, only 10/15 of the remaining patients received a second dose of ASCs according to the study protocol because five patients were discontinued from the study. Three of the discontinued patients presented with an almost-complete closure of the fistula, with a decrease in drainage of more than 50%; one patient presented with partial closure; and one patient presented with no change. Nine of the patients who received a second dose of ASCs completed the 8-week evaluation visit, and of these, one patient (11.1%)

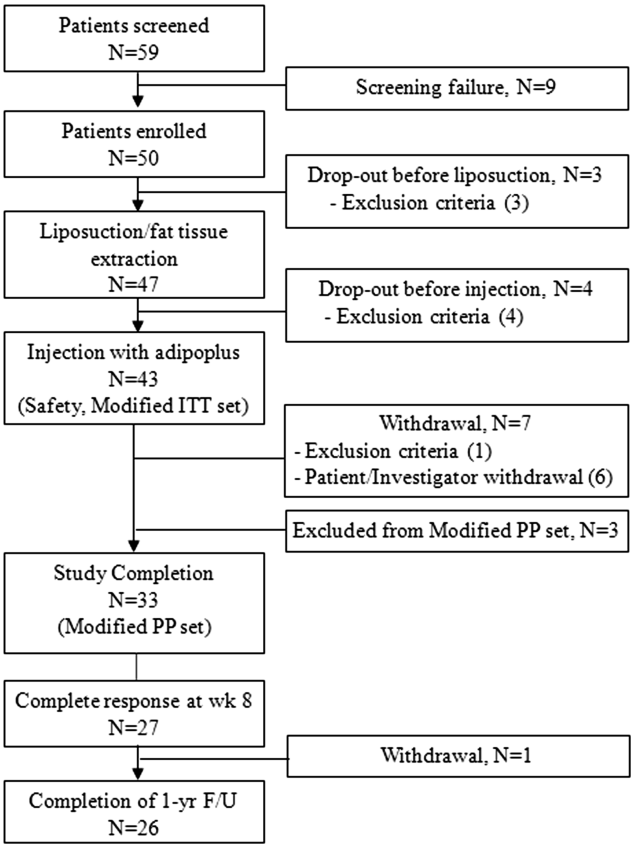


**Figure 1.** Patient disposition. Abbreviations: ITT, intention-to treat; PP, per-protocol.

showed complete healing of the fistula. Overall, incomplete healing was observed in 14 patients (33.3%) in the ITT analysis. Of these, only one patient showed no change and most of them (13/14) showed considerable decrease of drainage from the fistula.

ITT population was stratified based on the duration of disease, duration of fistula, and number of injected cells to assess whether some patients may have a clinical benefit from this procedure as compared to others (Table 3). There was no significant correlation between these parameters and efficacy.

### Sustain of Healing

Healing of fistula was sustained in 23 patients (88.5%) at month 12 out of 26 patients who were completed 1-year follow-up study (Fig. 2). One patient missed at month 12 visit was sustained complete closure of fistula up to month 9. Therefore, out of 27 patients, recurrence was observed in three patients at month 4, month 6, and month 9, respectively. Even recurrence of fistula, the patients still showed marked decrease of drainage and improved quality of life.

### Safety

The safety analysis included data from all 43 patients who received at least one dose of ASCs.

The most common AEs were postoperative pain, anal pain, and anal bleeding. These AEs affected 60%, 19%, and 7% of the patients, respectively. Postoperative pain was reported by patients upon recovery from the anesthetic mainly due to suturing of the internal opening of fistula tract or insertion of the anoscope used in the procedure. All postoperative pain was resolved with recovery of operation site and was determined by the investigators to be unrelated to ASC administration. No ASCs-related AEs were observed, and no grade 3 or 4 AEs occurred during the study period.

One individual (2% of the patients) was hospitalized due to a vitamin B12 deficiency. Based on the protocol, all hospitalizations must be reported as SAEs, regardless of the toxicity grade or the relationship to ASC administration. Therefore, this event was reported as an SAE despite a toxicity grade of 2 and an investigator assessment showing that the SAE was unrelated to the injection of ASCs. The SAE resolved with treatment.

During the additional observation study, two patients experienced grade 3 or 4 AEs. One of these events was exacerbation of disease of grade 3 and the other event corresponded to a grade 4 peritonitis due to regional enteritis caused by Crohn's disease, which were found to be unrelated to ASC administration and recovered with treatment. No occurrences of new fistulae related to the target fistula were reported during this study.

## Discussion

Symptomatic fistula from Crohn's disease is a devastating complication leading to major negative impact to the quality of life of patients.

In our phase II study, 79% of patients (26/33) showed complete closure of fistula after first dose injection which was notably superior to the result (46%) reported by Garcia-Olmo et al., although a direct comparison is not possible due to the different patient population; patients with Crohn's fistula in this study but patients with complex fistula including small

**Table 1.** Demographic and baseline characteristics

| Parameter | | Modified PP set ($N = 33$) | Modified ITT set ($N = 43$) |
|---|---|---|---|
| Gender ($n$, %) | Male, $n$ (%) | 22 (66.7) | 30 (69.8) |
| | Female, $n$ (%) | 11 (33.3) | 13 (30.2) |
| Age (years) | Mean ± SD | 26.7 ± 5.6 | 26.2 ± 5.4 |
| | Min – Max | 18–40 | 18–40 |
| Number of fistula | One | 22 (66.7) | 27 (62.8) |
| | Two | 8 (24.2) | 12 (27.9) |
| | More than Three | 3 (9.1) | 4 (9.3) |
| Type of fistula | Trans-sphincteric, $n$ (%) | 24 (72.7) | 30 (69.8) |
| | Suprasphincteric, $n$ (%) | 4 (12.1) | 5 (11.6) |
| | Intersphincteric, $n$ (%) | 0 (0.0) | 0 (0.0) |
| | Extrasphincteric, $n$ (%) | 5 (15.2) | 8 (18.6) |
| Duration of Crohn's disease (month) | 0–12, $n$ (%) | 5 (15.2) | 6 (14.0) |
| | 13–24, $n$ (%) | 5 (15.2) | 5 (11.6) |
| | 25–36, $n$ (%) | 2 (6.1) | 5 (11.6) |
| | 37–48, $n$ (%) | 3 (9.1) | 4 (9.3) |
| | $\gg$ 49, $n$ (%) | 18 (54.5) | 23 (53.5) |
| Duration of fistula (month) | 0–12, $n$ (%) | 6 (18.2) | 10 (23.3) |
| | 13–24, $n$ (%) | 7 (21.2) | 8 (18.6) |
| | 25–36, $n$ (%) | 0 (0.0) | 1 (2.3) |
| | 37–48, $n$ (%) | 5 (15.2) | 6 (14.0) |
| | $\gg$ 49, $n$ (%) | 15 (45.5) | 18 (41.9) |
| History of operation for fistula | Yes, $n$ (%) | 22 (66.7) | 26 (60.5) |
| | No, $n$ (%) | 11 (33.3) | 17 (39.5) |
| Medical history/Concomitant medication for Crohn's disease or fistula | Yes, $n$ (%) | 31 (93.9) | 41 (95.3) |
| | No, $n$ (%) | 2 (6.1) | 2 (4.7) |
| Diameter of target fistula | $\leq$1cm, $n$ (%) | 24 (72.7) | 30 (69.8) |
| | 1 cm $< d \leq$ 2 cm, $n$ (%) | 9 (27.3) | 13 (30.2) |
| Length of target fistula (cm) | Mean ± SD | 4.5 ± 1.6 | 4.6 ± 1.6 |
| | Min – Max | 3.0–9.0 | 2.7–9.0 |
| Number of injected cell at first ($\times 10^7$ cells) | Mean ± SD | 14.7 ± 7.8 | 15.8 ± 8.8 |
| | Min – Max | 9.0–42.0 | 9.0–42.0 |
| Number of injected cell at second ($\times 10^7$ cells) | $N$ | 7 | 10 |
| | Mean ± SD | 19.9 ± 5.2 | 19.1 ± 5.0 |
| | Min – Max | 13.5–27.0 | 13.5–27.0 |

Abbreviations: ITT, intention-to treat; PP, per-protocol.

group of Crohn's fistula in the other group [23]. A remarkable efficacy in this study might be mainly due to the injection amount of ASCs which was determined relative to the fistula size ($3 \times 10^7$ cells per 1 cm length of fistula in case of the diameter less than 1 cm). As a result, the average number of injected cells in the first injection was $15.8 \times 10^7$, whereas Garcia-Olmo group administered $2.0 \times 10^7$ ASCs, regardless of fistula size. Furthermore, we filled fistula tract with the mixture of a part of ASCs and fibrin glue instead of fibrin glue only. It has been reported that fibrin glue can increase cell transplant survival and enhance biological function of transplanted cells [27]. Similarly, ASCs in fibrin matrix was survived from the apoptotic condition and continuously released growth factors for 2 weeks during in vitro culture without supplement of nutrients (data not shown, in submission). Moreover, they successfully inhibited both T-cell proliferation and secretion of proinflammatory cytokines from the activated T cells (data not shown, in submission). It seems to be speculated, therefore, that ASCs with fibrin matrix might provide the additional effect on the fistula healing together with ASCs injected into the submucosa of fistula tract by affecting continuously to the lesion area in fistula tract.

**Table 2.** Efficacy outcome of phase II study

| | Modified PP set | Modified ITT set |
|---|---|---|
| | $N$ (%) | $N$ (%) |
| Number of patients | 33 | 43 |
| Patients with first injection | 33 | 42 |
| Complete closure | 26 (78.8) | 27 (64.3) |
| Closure more than 50% | 6 (18.2) | 14 (33.3) |
| Patients with second injection | 7 | 9 |
| Complete closure | 1 (14.3) | 1 (11.1) |
| Closure more than 50% | 5 (71.4) | 7 (77.8) |
| Overall closure after treatment | 27/33 (81.8) | 28/42 (66.7) |

Abbreviations: ITT, intention-to treat; PP, per-protocol.

Of note, ASC-mediated fistula healing was well-sustained for 1-year without recurrence. Twenty-three out of 26 patients (88.5%) with the complete closure of fistula at week 8 showed the sustained healing with epithelization of external opening. Given that recurrence is very common issue in patients with Crohn's fistula, our finding has important implication. Although infliximab treatment provides new option for the management of Crohn's fistula, the high recurrence during treatment or even maintenance therapy requires better medical approach. In the clinical study with infliximab, recurrence was more than 50% and 36% of the patients maintained complete closure at week 54 with maintenance therapy [28]. Sustained response in this study is similar to that of Spanish group in which 11.8% (2/17) of recurrence was observed after 1-year follow-up [29]. The Italian group, Ciccocioppo et al. also reported therapeutic potential of stem cell treatment with durable response [17]. They carried out clinical trial with BM-MSCs to treat the Crohn's fistula by injecting cells to

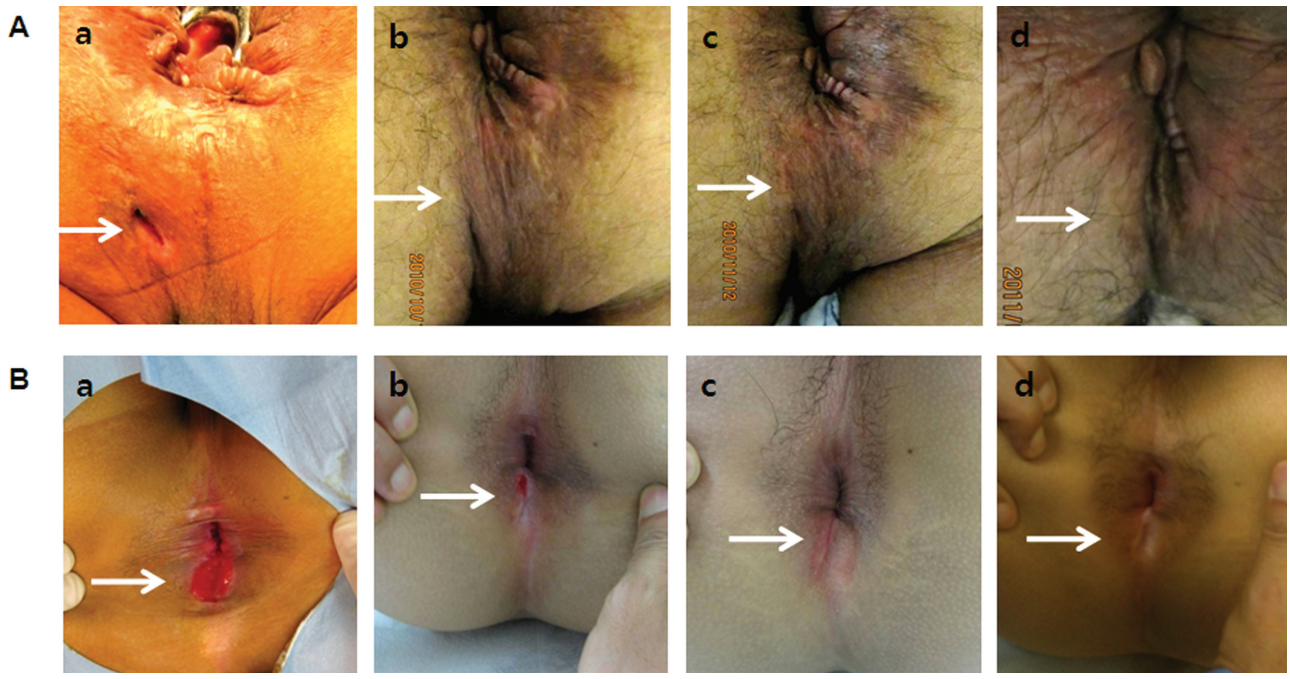


**Figure 2.** Patient **A, B**. Treatment with adipose tissue-derived stem cells. (a): Before injection; (b): at week 4 after injection; (c): at week 8 after injection; (d): at month 12 after injection, showing healed fistula with complete epithelialization of the external opening. White arrow: opening before injection or injected site.

intrafistula tract serially. BM-MSCs treatment induced a sustained closure of fistula tract with a reduction of Crohn's disease and perianal disease activity indexes, suggesting that local injection of BM-MSCs may affect regulation of systemic immune system as well as local site.

**Table 3.** Subgroup analysis of patients treated with ASCs (modified ITT set, $N = 42$)

| Parameter | | Complete Closure $n/N$ (%) | Pearson correlation coefficient ($R$) |
|---|---|---|---|
| Duration of Crohn's disease (month) | 0–12 | 3/6 (50.0) | −0.176 |
| | 13–24 | 5/5 (100.0) | |
| | 25–36 | 0/5 (0.0) | |
| | 37–48 | 3/4 (75.0) | |
| | ≫ 49 | 17/22 (77.3) | |
| Duration of fistula (month) | 0–12 | 4/10 (40.0) | −0.210 |
| | 13–24 | 6/8 (75.0) | |
| | 25–36 | 1/1 (100.0) | |
| | 37–48 | 5/6 (83.3) | |
| | ≫ 49 | 12/17 (70.6) | |
| Number of injected cells ($\times 10^7$ cells) | 9.0 | 7/10 (70.0) | 0.058 |
| | 10.5 | 1/3 (33.3) | |
| | 12.0 | 9/12 (75.0) | |
| | 13.5 | 1/1 (100.0) | |
| | 15.0 | 3/5 (60.0) | |
| | 18.0 | 0/1 (0.0) | |
| | 21.0 | 1/1 (100.0) | |
| | 24.0 | 2/2 (100.0) | |
| | 27.0 | 1/2 (50.0) | |
| | 30.0 | 0/1 (0.0) | |
| | 39.0 | 1/1 (100.0) | |
| | 42.0 | 1/2 (50.0) | |
| | 45.0 | 1/1 (100.0)[a] | |

[a]Number of injected cells of this patient was presented as a sum of first and second treatment.

Long-term efficacy of ASCs in our study might be related to their immunomodulatory ability. Several studies have now shown that ASCs are capable of regulating the functions of a diverse array of cells in the immune system [30–32]. ASCs can interact with B lymphocytes, T lymphocytes, NK cells, monocyte-derived dendrite cells, and neutrophils, affecting differentiation and activation of immune cells [33–38]. ASCs also can release various cytokines related to suppress immune response such as TGF $\beta$, IL-10, prostaglandin E2, indoleamine 2,3 dioxygenase, and nitric oxide [39–43]. Indeed, we found that ASCs significantly inhibited secretion of proinflammatory cytokines such as TNF-$\alpha$ and IFN-$\gamma$ from activated peripheral blood mononuclear cells (data not shown, in preparation). Given that ASC treatment is considered to induce optimal balance of immune system, resulting in healing of fistula, subsequently, it may be feasible to consider that rebalanced immune system may lead to sustained response. Italian group also found that regulatory T cells increased by MSCs treatment remained stable until 1-year follow-up.

The optimal therapeutic goals for the treatment of Crohn's fistulae are complete healing of the lesions, a sustained effect of the administered ASCs without recurrence, and no damage to the anal sphincter. In this context, our findings suggest that ASCs therapy could be promising treatment option for the patients with Crohn's fistula refractory to the conventional therapy.

## Conclusion

ASCs treatment, with dose scheme proportional to fistula's surface area, produced a favorable therapeutic outcome, good tolerability, and safety in the patients with Crohn's fistula for longer than 1-year.

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## Disclosure of Potential Conflicts of Interest

Dr. Yu, Dr. Park, Dr. Cho, Dr. Lee, Dr. Song, Dr. Kim, Dr. Jeong, and Dr. Ha do not have a potential or real conflict of interest. Dr. Kim. MS. Kim, and Dr. Yoo are employees of sponsor.

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