



Long-Term Results of Adipose-Derived Stem Cell Therapy for the Treatment of Crohn's Fistula

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ABSTRACT

A previous phase II clinical trial of adipose-derived stem cell (ASC) therapy for fistulae associated with Crohn's disease, a devastating condition with a high recurrence rate, demonstrated safety and therapeutic potential with a 1-year sustained response. In the present study, 41 of the 43 phase II trial patients were followed for an additional year, regardless of response in the initial year. At 24 months, complete healing was observed in 21 of 26 patients (80.8%) in modified per protocol analysis and 27 of 36 patients (75.0%) in modified intention-to-treat analysis. No adverse events related to ASC administration were observed. Furthermore, complete closure after initial treatment was well-sustained. These results strongly suggest that autologous ASCs may be a novel treatment option for Crohn's fistulae. *STEM CELLS TRANSLATIONAL MEDICINE 2015;4:1-6*

SIGNIFICANCE

Long-term follow-up of patients with Crohn's fistula found that one or two doses of autologous adipose-derived stem cell therapy achieved complete closure in 80% of the patients at 12 months. After 24 months, 75% of those patients sustained complete closure, showing sustainable safety and efficacy of the treatment.

INTRODUCTION

Crohn's disease is a life-long inflammatory disease with clinical symptoms such as abdominal pain, diarrhea, nausea, fever, and vomiting [1]. Crohn's disease may involve a wide range of the gastrointestinal tract from the mouth to the anus; it results in a chronic autoimmune disorder in which the body's immune system attacks the gastrointestinal tract. Inflammation caused by Crohn's disease demonstrates a typical transmural characteristic but is not superficially confined to the mucosa or submucosa of the intestine. Chronic inflammation in Crohn's patients can extend completely through the intestinal wall and create a fistula, which is an abnormal connection between the intestine and another organs or skin.

Crohn's fistula is one of the most distressing diseases because it decreases patient's quality of life and frequently recurs [2]. It has been reported to occur in 13%–38% of patients with Crohn's disease, and a proctectomy is required in 10%–18% of Crohn's patients over the course of the disease [3, 4].

Several classifications and a scoring system to evaluate disease severity have been developed

to determine optimal management strategy. From an anatomical point of view, there are two types of anal fistulae: high and low. The former involves the anal sphincter or very high locations that are hard to access, making it difficult to operate because of the high risk of developing incontinence, whereas for the latter, many surgical options exist, such as fistulotomy and fistulectomy, with less concern of fecal incontinence [4–7]. Nevertheless, surgical intervention continues to be problematic with high rates of recurrence and short-term efficacy [8, 9].

Although antibiotics are usually used as a first-line therapy for fistula healing, they have not been proven effective in healing perianal Crohn's fistula [10]. Uncontrolled or controlled studies have suggested that antibiotics, such as metronidazole and ciprofloxacin, may be of short-term benefit in the closure of Crohn's fistula [11, 12]. In a meta-analysis of 5 studies, a response was found in 54% of the patients treated with azathioprine or 6-mercaptopurine, but in only 21% of the placebo group [13]. However, this meta-analysis is limited in that literatures reviewed are not well-designed prospective clinical studies, and response evaluation for the fistula was made with different criteria, with

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complete closure or decreased drainage as a secondary endpoint. With clinical evidence, though the result is not satisfactory, biological agents, such as anti-tumor necrosis factor (TNF)- α monoclonal antibody, are increasingly used to treat Crohn's fistula [5, 14, 15]. In the long-term treatment study of infliximab (ACCENT II study), the closure of fistula after the initial three infusions occurred in 63% of patients at week 14 but decreased to 36% at week 54, although additional therapy was given every 8 weeks [16]. Further, there is concern of possible adverse events (AEs) with long-term maintenance therapy, such as occurrence of infectious complications related to impairment of the patient's immune system, development of abscess, and recurrence of the fistula tract. Overall, the treatments currently available for Crohn's fistula remain unsatisfactory because they fail to achieve complete closure, lower recurrence, and limit adverse effects [5, 17].

Given the challenges and unmet medical needs in Crohn's fistula, attention has been directed at stem cell therapy. Several studies suggest that mesenchymal stem cells (MSCs) improve Crohn's disease and Crohn's fistula [18–20]. The Ciccocioppo group [20] demonstrated that locally injected autologous MSCs derived from bone marrow have therapeutic efficacy without adverse reaction in the treatment of fistulizing Crohn's disease, suggesting that MSCs may modulate the activity of mucosal T cells in the inflamed intestine. A number of clinical trials for refractory Crohn's disease have also evaluated autologous or allogenic MSCs and have shown that MSCs can be safely administered by intravenous infusion with some patients achieving clinical response [18, 19].

Our previous phase I and II clinical trials strongly demonstrated that MSCs derived from adipose tissue (ASCs) are a safe and useful therapeutic tool for the treatment of Crohn's fistula with favorable efficacy and complete healing in 82% of the patients [21, 22]. The purpose of this retrospective study was to evaluate the long-term outcome of ASC therapy in Crohn's fistula.

MATERIALS AND METHODS

Patients

Eligible patients came from our phase II study in which we examined the safety and efficacy of one or two ASC injections into the tract of fistulae associated with Crohn's disease. The phase II clinical trial was carried out for a term of 1 year at 5 hospitals in South Korea from January 2010 to August 2012. All patients have now passed the 2-year window from the initiation of the phase II study.

Preparation of ASCs

ASCs were isolated from lipoaspirates of each patient's subcutaneous fat tissue as described previously [22]. Fat tissue (10–40 ml) was digested in phosphate-buffered saline (HyClone, Logan, UT, <http://www.hyclone.com>) 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 (bFGF) to obtain the required number of ASCs for injection. ASCs were subcultured to passage 3–4. After harvesting via trypsinization, cells were suspended in DMEM and packaged into single-use vials containing 3×10^7 cells/ml. All manufacturing procedures were carried out according to the good manufacturing practices authorized by the Korean Food

and Drug Administration. For lot release testing, ASCs were assessed for cell appearance, viability, identification, purity, content, and potency. The 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 endotoxin units per milliliter.

Administration of ASCs

Before injection with ASCs, the fistula tract was thoroughly cultered and irrigated under anesthesia. After the internal opening was completely sutured using 2-0 vicryl, cells were evenly injected into the submucosa surrounding the internal opening and along the fistula tract wall. Open fistula tract was filled with a mixture of ASCs and fibrin glue (kit from Green Plast, Seoul, Korea, <http://greenplast.lookchem.com>; or Tisseel-fibrin sealant from Baxter Healthcare, Vienna, Austria, <http://www.baxterhealthcare.com>) using a dual-syringe injection system. A maximum of 30% of ASCs for the injection was mixed with fibrin glue. The dose of ASCs was determined based on the fistula size. The fistula size was determined from the diameter and length of the fistula, which were measured using a probe before injection. Approximately 3×10^7 cells per centimeter of length were injected when the diameter of the fistula was not above 1 cm, and twice that number of cells was administered when the diameter of the fistula was $1 \text{ cm} < d \leq 2 \text{ cm}$. If a second injection was necessary, 1.5 times the number of cells from the first injection was used.

Study Design

For this retrospective analysis, patient information was collected by reviewing medical records from the internal medicine and surgery divisions to evaluate the sustainability of the efficacy and assess any adverse effect related to the stem cell therapy. Data collection included AEs, fistula healing, surgery, and infliximab treatment caused by recurrence. The study was conducted according to good clinical practice guidelines and the principles set out in the Declaration of Helsinki. The study protocol was approved by institutional review boards at each study site.

Assessments

In this observational study, fistula healing was evaluated 24 months after the administration of ASCs. Fistula healing was defined as complete closure of the fistula tract, including internal and external openings, without drainage or any sign of inflammation. Safety evaluations included systemic tolerance, AEs, and serious AEs. The patients who received surgical treatment and infliximab therapy during follow-up are described.

Statistical Analysis

Efficacy was assessed by modified intention-to-treat (mITT) analysis and modified per protocol (mPP) analysis. The mPP analysis was performed for the patients who were included in the mPP group in the phase II study and had efficacy data at month 24. During this follow-up study, patients who received other surgical procedures or operations involving the injection site were excluded from the mPP analysis. The mITT analysis included the patients who received ASC treatment and had efficacy data at month 24. Patients discontinuing the study were included in the efficacy analysis until the time of their discontinuation. For safety analysis,

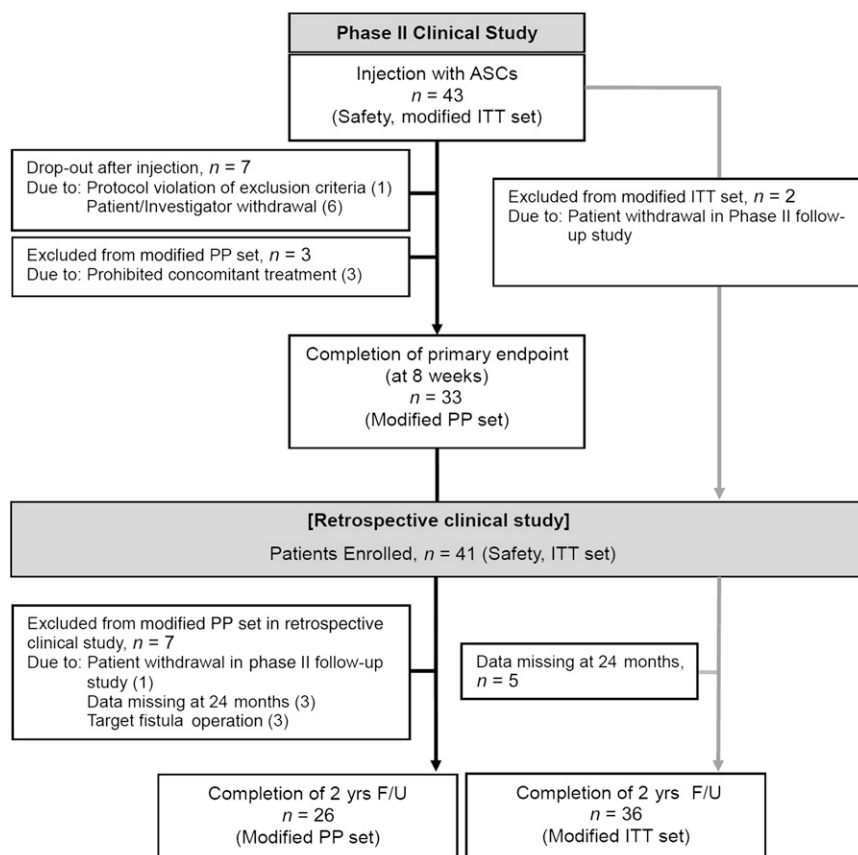


Figure 1. Patient disposition. Abbreviations: ASC, adipose-derived stem cell; F/U, follow-up; ITT, intention-to-treat; PP, per protocol; yrs, years.

an intention-to-treat analysis was performed based on initial treatment intent and a record of at least one visit during follow-up.

RESULTS

Study Population

In the initial phase II study, 43 patients received ASC injections. Of these, 41 were included in the ITT analysis (Fig. 1). Of these, 68.3% ($n = 28$) were male, and 31.7% ($n = 13$) were female. The mean age was 26.2 ± 5.5 years. The average duration of Crohn's disease was 58.0 ± 40.0 months, and the average duration of fistula was 48.2 ± 42.2 months. The mean fistula length was 4.6 ± 1.6 cm. The type of fistula was trans-sphincteric in 28 patients (68.3%), extrasphincteric in 8 patients (19.5%), and suprasphincteric in 5 patients (12.2%). The average ASC injection volume was 5.5 ± 2.8 ml per lesion site, and the average number of injected cells was 16.4×10^7 (Table 1). Among the 33 patients in the mPP group of the phase II study, 26 were included in the mPP analysis in this retrospective study. Three patients were excluded because of target fistula operation, including two cases of seton placement and one case of fistulotomy after the month 12 visit. Four other patients were excluded because of data missing at month 24. Of the 41 patients included in the ITT set, 4 patients (9.8%) required surgical management because of recurrence of the target fistula (a seton was placed in three cases, and fistulotomy was performed in one case), and eight patients (19.5%) received infliximab because of exacerbation of enteric Crohn's disease (Table 2).

Two-Year Outcome of ASC Therapy

Efficacy is shown in Table 3. In the mPP analysis, 79.3% (23 of 29) and 80.8% (21 of 26) of the patients showed complete fistula healing at month 12 and month 24 after ASC injection, respectively. In the mITT analysis, 80.0% (28 of 35) and 75.0% (27 of 36) of the patients showed a complete response at month 12 and month 24, respectively. Only 9 patients (25.0%) demonstrated an incomplete response at month 24.

Sustained Response

To evaluate the durability of the initial response to ASC therapy, 27 patients who showed complete closure at week 8 after ASC injection were analyzed. Of these, 24 patients were included in this study, and 20 of them (83.3%) still showed complete closure at year 2, whereas 4 patients presented a recurrence (Table 4). Of these 4 recurrences, 3 occurred within 1 year of treatment, and 1 occurred after 1 year.

Consideration of Anti-TNF- α Inhibitor

Among the 27 patients who showed complete healing at 24 months in the mITT group, four patients received the anti-TNF- α inhibitor infliximab. Two patients received infliximab during the phase II study, whereas the other patients received infliximab after 1 year of follow-up. In all cases, infliximab treatment was prescribed by a gastroenterologist because of exacerbation of enteric Crohn's disease. In addition, three of the four cases presented complete closure before infliximab treatment and sustained fistula healing after infliximab. This suggests that infliximab does not appear to affect the long-term efficacy of ASC therapy.

Table 1. Patient parameters

Parameter	ASC injection (n = 41)
Gender	
Male, n (%)	28 (68.3)
Female, n (%)	13 (31.7)
Age (year)	
n	41
Mean ± SD	26.2 ± 5.5
Min–Max	18–40
Duration of Crohn's disease (months)	
n	33
Mean ± SD	58.0 ± 40.0
Min–Max	9–135
Duration of fistula (months)	
n	33
Mean ± SD	48.2 ± 42.2
Min–Max	2–175
Diameter of target fistula (cm)	
n	41
Mean ± SD	1.0 ± 0.5
Min–Max	0.3–2.0
≤1 cm, n (%)	28 (68.3)
1 cm < d ≤ 2 cm, n (%)	13 (31.7)
Length of target fistula (cm)	
Mean ± SD	4.6 ± 1.6
Min–Max	2.7–9.0
Volume of cells injected (ml) ^a	
Mean ± SD	5.5 ± 2.8
Min–Max	3.0–14.0
Number of cells injected ^a	
Mean ± SD	16.4 ± 8.4
Min–Max	9.0–42.0
Volume of mixture of ASCs and fibrin glue injected ^a	
Mean ± SD	4.1 ± 2.0
Min–Max	2.0–10.0

^aThe value was calculated by including first and second injection volumes.

Abbreviations: ASC, adipose-derived stem cell; Max, maximum; Min, minimum.

Safety

During this study period, all available safety data were collected from 41 patients. A total of 53 adverse events were reported in 30 patients (73.2%), and the most common AEs were abdominal pain (17.1%); eczema and exacerbation of disease (each 9.8%); and anal inflammation, diarrhea, and fever (each 7.3%). ASC-related AEs were not observed.

DISCUSSION

Despite many decades of research, adequate therapy is lacking for fistulae derived from Crohn's disease, and the condition remains an unmet medical need. In the mPP analysis of the phase II study evaluating the efficacy and safety of ASCs, 82% of the patients (27 of 33) showed complete closure of fistula after the final injection

Table 2. Treatments during follow-up

Detailed description	n (%) ^a
Surgical procedure	
Seton	3 (7.3)
Anal fistulotomy	1 (2.4)
Infliximab treatment because of exacerbation of enteric Crohn's disease	8 (19.5)

^aThe value was calculated based on the intention-to-treat population.

Table 3. Efficacy of treatments

Efficacy parameter	12 months		24 months	
	Modified PP set (n = 29)	Modified ITT set (n = 35)	Modified PP set (n = 26)	Modified ITT set (n = 36)
Complete closure	23 (79.3)	28 (80.0)	21 (80.8)	27 (75.0)
Incomplete closure	6 (20.7)	7 (20.0)	5 (19.2)	9 (25.0)
p value ^a	.0003 ^b	<.0001 ^b	<.001 ^b	<.001 ^b

^aChi-square test.

^bStatistically significant difference.

Abbreviations: ASC, adipose-derived stem cell; ITT, intention-to-treat; Max, maximum; Min, minimum; PP, per protocol.

Table 4. Long-term efficacy of treatments

	Week 8	Month 12	Month 24
Maintenance of complete closure	27	23 of 26 (88.5%)	20 of 24 (83.3%)
Recurrence		3 of 26 (11.5%)	4 of 24 (16.7%)
Description of missing patients		1 case: patient withdrawal at month 12	1 case: patient withdrawal at month 12 2 cases: data missing at month 24

at week 8 of evaluation, and durability of response was observed in 23 of 26 patients at month 12. In this retrospective additional year of observation, 20 of 24 patients (83.3%) showed sustained complete response through month 24.

In the modified ITT analysis, 80.0% (28 of 35) and 75.0% of the patients (27 of 36) showed complete response at 12 and 24 months, respectively. We believe these data are promising, considering the major problem of recurrence after other therapies such as infliximab [23–26].

The sustained efficacy observed in this study compared with other stem cell therapy is also remarkable [27]. A previous study conducted by a Spanish group using ASCs to treat complex perianal fistulae demonstrated favorable therapeutic efficacy with a healing rate as high as 71% in their phase II clinical trial; however, a randomized phase III clinical trial failed to demonstrate statistically significant efficacy. In addition, in a long-term retrospective follow-up study extending phase II, the recurrence of fistulae was observed in a considerable portion of the study population, in which only 7 of 12 initial responders sustained complete closure [27, 28].

The present study is different from that of the Spanish group in its clinical design and in the characteristics of the stem cells expanded in vitro. From a clinical point of view, this study was limited

to complex fistulae originating from Crohn's disease, whereas the other two studies focused mainly on the complex cryptoglandular fistulae; only a small number of Crohn's patients was included in the phase II study. The two types of fistula have a similar pathophysiology but different etiologies. The cryptoglandular fistula is involved in cryptoglandular infections leading to abscess formation, whereas Crohn's fistula is mainly caused by an abnormally activated immune system resulting in chronic inflammation and deep lesion. In this regard, ASCs appear to be beneficial for Crohn's fistulae rather than fistulae of cryptoglandular origin. This may be explained by the fact that ASCs have a potent immunomodulatory function: they inhibit T-cell proliferation and proinflammatory cytokine production from activated immune cells.

In addition, there is a substantial difference in dosage schemes. We designed a dosage proportional to the fistula size, not a fixed dosage. Fistula size in our phase II clinical study had a wide range of length and diameter, 2.7–9.0 and 0.3–2.0 cm, respectively, resulting in administered cell numbers of $9.0\text{--}42.0 \times 10^7$ cells. Thus, we suspect that the cell volume injected into the fistula tract was adequate for rebalancing the local immunological disorder. Another important aspect is that the fistula tract was filled with a mixture of fibrin glue and ASC. Many Crohn's fistulae have some part difficult to inject technically because they are located in a high position, and even the injected ASCs tend to reflux into the tract because the submucosa of the fistulous tract is very soft from chronic inflammation. The ASCs injected with fibrin glue may have a long-term synergistic effect with cells injected under the subepithelial layer of the fistula tract. ASCs in fibrin glue matrix effectively release growth factors to enhance wound healing and soluble factors to inhibit immune response by suppressing both T-cell proliferation and secretion of proinflammatory cytokines from the activated T cells, although cells in fibrin glue matrix may not migrate to the tissue [29]. In addition, the ASCs used in our clinical trial may have different characteristics than those of other clinical trials, even if MSCs were harvested from the same adipose tissue.

Accumulating evidence suggests that the *ex vivo* expansion of MSCs might affect clinical results because various culture conditions, including culture media, addition of supplementary factors, and even cell plating density, influence the characteristics of MSCs [30–32]. In particular, the use of bFGF as a supplementary factor greatly increases the proliferation rate and the *in vivo* immunosuppressive potential [30, 33], which we also observed (data not shown). Thus, it is likely that the clinical study design and ASC culture method are two important factors that might affect therapeutic efficacy in clinical trials.

Although there are many surgical options, such as simple fistulotomy, fistulectomy, seton placement, or advancement flap, surgical management is not recommended for complex fistulae associated with anal sphincter muscle or with tracts above the anal sphincter because of the high risk of anal incontinence. Incontinence may also develop in patients who undergo multiple surgeries for complex and recurrent abscesses or fistulae.

Pharmacological treatment for remission of inflammation is important to improve outcome. The anti-TNF- α antibody infliximab, regarded as a promising treatment option, has not provided a satisfactory long-term outcome, instead presenting a high recurrence rate [5, 23–26]. In the clinical study with infliximab, recurrence was greater than 50% within 3 months after cessation of treatment, and 36% of the patients were found to have complete

closure at week 54 with maintenance therapy [16]. This indicates that a substantial number of patients may still suffer from draining fistulae, even when treated with a long-term regimen. Thus, it is noteworthy that ASC-mediated fistula healing was well-sustained for 2 years without any clinically significant recurrence rate, even with only one or two doses. This has important implications for physicians and patients with Crohn's fistula.

The sustained efficacy of ASCs in our study may be related to their immunomodulatory ability [34–36]. Accumulating evidence demonstrates that MSCs are able to induce immunosuppression by regulating the proliferation and function of a variety of immune cells, as well as by generating regulatory T cells [37–39]. Given that Crohn's disease involves immune dysregulation characterized by chronic and recurrent inflammation with increasing proinflammatory cytokine levels secreted by activated immune cells in the intestine, ASCs injected in the lesions of Crohn's patients can readily be activated to exert their immunosuppression activity and induce peripheral tolerance. It is therefore likely that ASC treatment helps to restore immune homeostasis, resulting in healing of fistulae and sustained response [20, 40].

The therapeutic goal in Crohn's fistula is to completely close the fistula without recurrence, or at least to suppress recurrence as much as possible. Unfortunately, Crohn's fistula healing is hardly ever achieved, and fistulae readily recur even after initial response to current treatments, even in cases of simple fistulae. ASCs represent a novel therapeutic option for Crohn's fistulae with a high risk of recurrence, showing durable efficacy with low recurrence, even in cases in which healing cannot be achieved with biologics or in which conventional surgical procedures cannot be performed. Such refractory patients should be referred to tertiary centers where optimal therapy, including stem cell implants, can be offered.

CONCLUSION

Long-term follow-up of patients with Crohn's fistula found that one or two doses of autologous ASC therapy achieved complete closure in 75% of the patients at 24 months and sustainable safety and efficacy of initial response in 83% of the patients.

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AUTHOR CONTRIBUTIONS

Y.B.C. and K.J.P.: manuscript writing, administrative support, and data analysis and interpretation; S.N.Y., K.H.S., D.S.K., and S.H.J.: administrative support and data analysis and interpretation; M.K. and H.Y.J.: conception and design, collection and assembly of data, and data analysis and interpretation; C.S.Y.: conception and design, administrative support, and final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

C.S.Y. and H.Y.J. are compensated employees of Anterogen Co. Ltd. The other authors indicated no potential conflicts of interest.

REFERENCES

- 1 Maconi G, Bolzoni E, Giussani A et al. Accuracy and cost of diagnostic strategies for patients with suspected Crohn's disease. *J Crohn's Colitis* 2014;8:1684–1692.
- 2 Sangwan YP, Schoetz DJ Jr., Murray JJ et al. Perianal Crohn's disease: Results of local surgical treatment. *Dis Colon Rectum* 1996;39:529–535.
- 3 Sandborn WJ, Fazio VW, Feagan BG et al. AGA technical review on perianal Crohn's disease. *Gastroenterology* 2003;125:1508–1530.
- 4 Hwang SH, Bang MJ. Surgery for a complex anal fistula. *J Korean Soc Coloproctol* 2008;24:77–82.
- 5 Gecse KB, Bemelman W, Kamm MA et al. A global consensus on the classification, diagnosis and multidisciplinary treatment of perianal fistulising Crohn's disease. *Gut* 2014;63:1381–1392.
- 6 Parks AG, Stitz RW. The treatment of high fistula-in-ano. *Dis Colon Rectum* 1976;19:487–499.
- 7 Kodner IJ, Mazor A, Shemesh EI et al. Endorectal advancement flap repair of rectovaginal and other complicated anorectal fistulas. *Surgery* 1993;114:682–689; discussion 689–690.
- 8 van Koperen PJ, Safiruddin F, Bemelman WA et al. Outcome of surgical treatment for fistula in ano in Crohn's disease. *Br J Surg* 2009;96:675–679.
- 9 Ritchie RD, Sackier JM, Hodde JP. Incontinence rates after cutting seton treatment for anal fistula. *Colorectal Dis* 2009;11:564–571.
- 10 Present DH. Crohn's fistula: Current concepts in management. *Gastroenterology* 2003;124:1629–1635.
- 11 Dejaco C, Harrer M, Waldhoer T et al. Antibiotics and azathioprine for the treatment of perianal fistulas in Crohn's disease. *Aliment Pharmacol Ther* 2003;18:1113–1120.
- 12 Thia KT, Mahadevan U, Feagan BG et al. Ciprofloxacin or metronidazole for the treatment of perianal fistulas in patients with Crohn's disease: A randomized, double-blind, placebo-controlled pilot study. *Inflamm Bowel Dis* 2009;15:17–24.
- 13 Pearson DC, May GR, Fick GH et al. Azathioprine and 6-mercaptopurine in Crohn disease. A meta-analysis. *Ann Intern Med* 1995;123:132–142.
- 14 Greenstein AJ, Kark AE, Dreiling DA. Crohn's disease of the colon: I. Fistula in Crohn's disease of the colon, classification presenting features and management in 63 patients. *Am J Gastroenterol* 1974;62:419–429.
- 15 Williams JG, MacLeod CA, Rothenberger DA et al. Seton treatment of high anal fistulae. *Br J Surg* 1991;78:1159–1161.
- 16 Sands BE, Anderson FH, Bernstein CN et al. Infliximab maintenance therapy for fistulizing Crohn's disease. *N Engl J Med* 2004;350:876–885.
- 17 Levy C, Tremaine WJ. Management of internal fistulas in Crohn's disease. *Inflamm Bowel Dis* 2002;8:106–111.
- 18 Liang J, Zhang H, Wang D et al. Allogeneic mesenchymal stem cell transplantation in seven patients with refractory inflammatory bowel disease. *Gut* 2012;61:468–469.
- 19 Duijvestein M, Vos AC, Roelofs H et al. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: Results of a phase I study. *Gut* 2010;59:1662–1669.
- 20 Ciccocioppo R, Bernardo ME, Sgarella A et al. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulizing Crohn's disease. *Gut* 2011;60:788–798.
- 21 Cho YB, Lee WY, Park KJ et al. Autologous adipose tissue-derived stem cells for the treatment of Crohn's fistula: A phase I clinical study. *Cell Transplant* 2013;22:279–285.
- 22 Lee WY, Park KJ, Cho YB et al. Autologous adipose tissue-derived stem cells treatment demonstrated favorable and sustainable therapeutic effect for Crohn's fistula. *STEM CELLS* 2013;31:2575–2581.
- 23 Sciaudone G, Di Stazio C, Limongelli P et al. Treatment of complex perianal fistulas in Crohn disease: Infliximab, surgery or combined approach. *Can J Surg* 2010;53:299–304.
- 24 Tozer PJ, Burling D, Gupta A et al. Medical, surgical and radiological management of perianal Crohn's fistulas. *Aliment Pharmacol Ther* 2011;33:5–22.
- 25 Van Assche G, Vanbeckvoort D, Bielen D et al. Magnetic resonance imaging of the effects of infliximab on perianal fistulizing Crohn's disease. *Am J Gastroenterol* 2003;98:332–339.
- 26 Taxonera C, Schwartz DA, Garcia-Olmo D. Emerging treatments for complex perianal fistula in Crohn's disease. *World J Gastroenterol* 2009;15:4263–4272.
- 27 Garcia-Olmo D, Herreros D, Pascual I et al. Expanded adipose-derived stem cells for the treatment of complex perianal fistula: A phase II clinical trial. *Dis Colon Rectum* 2009;52:79–86.
- 28 Guadalajara H, Herreros D, De-La-Quintana P et al. Long-term follow-up of patients undergoing adipose-derived adult stem cell administration to treat complex perianal fistulas. *Int J Colorectal Dis* 2012;27:595–600.
- 29 Kim I, Lee SK, Yoon JI et al. Fibrin glue improves the therapeutic effect of MSCs by sustaining survival and paracrine function. *Tissue Eng Part A* 2013;19:2373–2381.
- 30 Sotiropoulou PA, Perez SA, Salagianni M et al. Characterization of the optimal culture conditions for clinical scale production of human mesenchymal stem cells. *STEM CELLS* 2006;24:462–471.
- 31 Cholewa D, Stiehl T, Schellenberg A et al. Expansion of adipose mesenchymal stromal cells is affected by human platelet lysate and plating density. *Cell Transplant* 2011;20:1409–1422.
- 32 Salem HK, Thiemermann C. Mesenchymal stromal cells: Current understanding and clinical status. *STEM CELLS* 2010;28:585–596.
- 33 Tsutsumi S, Shimazu A, Miyazaki K et al. Retention of multilineage differentiation potential of mesenchymal cells during proliferation in response to FGF. *Biochem Biophys Res Commun* 2001;288:413–419.
- 34 Le Blanc K. Mesenchymal stromal cells: Tissue repair and immune modulation. *Cytotherapy* 2006;8:559–561.
- 35 Yagi H, Soto-Gutierrez A, Parekkadan B et al. Mesenchymal stem cells: Mechanisms of immunomodulation and homing. *Cell Transplant* 2010;19:667–679.
- 36 Yoo KH, Jang IK, Lee MW et al. Comparison of immunomodulatory properties of mesenchymal stem cells derived from adult human tissues. *Cell Immunol* 2009;259:150–156.
- 37 Ge W, Jiang J, Arp J et al. Regulatory T-cell generation and kidney allograft tolerance induced by mesenchymal stem cells associated with indoleamine 2,3-dioxygenase expression. *Transplantation* 2010;90:1312–1320.
- 38 Gonzalez-Rey E, Gonzalez MA, Varela N et al. Human adipose-derived mesenchymal stem cells reduce inflammatory and T cell responses and induce regulatory T cells in vitro in rheumatoid arthritis. *Ann Rheum Dis* 2010;69:241–248.
- 39 Batten P, Sarathchandra P, Antoniw JW et al. Human mesenchymal stem cells induce T cell anergy and downregulate T cell allo-responses via the TH2 pathway: Relevance to tissue engineering human heart valves. *Tissue Eng* 2006;12:2263–2273.
- 40 González MA, Gonzalez-Rey E, Rico L et al. Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. *Gastroenterology* 2009;136:978–989.