ORIGINAL CONTRIBUTION

Autologous Expanded Adipose-Derived Stem Cells for the Treatment of Complex Cryptoglandular Perianal Fistulas: A Phase III Randomized Clinical Trial (FATT 1: Fistula Advanced Therapy Trial 1) and Long-term Evaluation

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ABSTRACT

BACKGROUND: Autologous adipose-derived stem cells may represent a novel approach for the management of complex fistula-in-ano. After successful phase I and II clinical trials, a phase III trial was performed to investigate the safety and efficacy.

DESIGN: In this multicenter, randomized, single-blind, add-on clinical trial, 200 adult patients from 19 centers were randomly assigned to receive 20 million stem cells (group A, 64 patients), 20 million adipose-derived stem cells plus fibrin glue (group B, 60 patients), or fibrin glue (group C, 59 patients) after closure of the internal opening. Fistula healing was defined as reepithelization of the external opening and absence of collection >2 cm by MRI. If the fistula had not healed at 12 weeks, a second dose (40 million stem cells in groups A and B) was administered. Patients were evaluated at 24 to 26 weeks (primary end point) and at 1 year (long-term follow-up).

RESULTS: All results are according to the "blinded evaluator" assessment. After 24 to 26 weeks, the healing rate was 39.1%, 43.3%, 37.3% in groups A, B, and C ($p = 0.79$). At 1 year, the healing rates were 57.1%, 52.4%, and 37.3 % ($p = 0.13$). On analysis of the subpopulation treated at the technique's pioneer center, healing rates were 54.55%, 83.33%, and 18.18%, at 24 to 26 weeks ($p < 0.001$). No SAEs were reported.

CONCLUSIONS: In treatment of complex fistula-in-ano, a dose of 20 or 60 million adipose-derived stem cells alone or in combination with fibrin glue was considered a safe treatment, achieving healing rates of approximately 40% at 6 months and of more than 50% at 1-year follow-up. It was equivalent to fibrin glue alone. No statistically significant differences were found when the 3 groups where compared. Clinical trials registration: www.clinicaltrials.gov, identifier NCT00475410; Sponsor, Cellerix SA.

KEY WORDS: Cryptoglandular; Fistula-in-ano; Perianal fistula; Adipose-derived stem cell; Stem cell therapy.

Financial Disclosures: Prof D. García-Olmo is Chairman of the UAM-Cellerix Cell Therapy and Regenerative Medicine Department, to which Cellerix has contributed €40,000 per year. UAM and Cellerix S.A. share patent rights to Cx401 (Adipose Derived Stem Cells). Prof D. García-Olmo is a member of the Advisory Board of Cellerix. M. Garcia-Arranz and Prof D. García-Olmo have applied for 2 patents related to Cx401 titled "Identification and isolation of multipotent cells from non-osteochondral mesenchymal tissue" (WO 2006/057649) and "Use of adipose tissue-derived stromal stem cells in treating fistula" (WO 2006/136244).

Poster presentation at the meeting of The American Society of Colon and Rectal Surgeons, Vancouver, BC, Canada, May 14 to 18, 2011.

Clinical trial registration at www.clinicaltrials.gov: ID NCT00475410, Efficacy and Safety of Adipose Stem Cells to Treat Complex Perianal Fistulas Not Associated to Crohn´s Disease (FATT1); and ID NCT01020825, Long-term Safety and Efficacy of Adipose-Derived Stem Cells to Treat Complex Perianal Fistulas in Patients Participating in the FATT-1 Randomized Controlled Trial (LTE).

The European Medicines Agency (EMA) granted Cx401 (Autologous Expanded Adipose-Derived Stem Cells (eASCs)) "Orphan Drug" status in 2005 for the treatment of anal fistula (Orphan Designation EU number EU/3/05/303).

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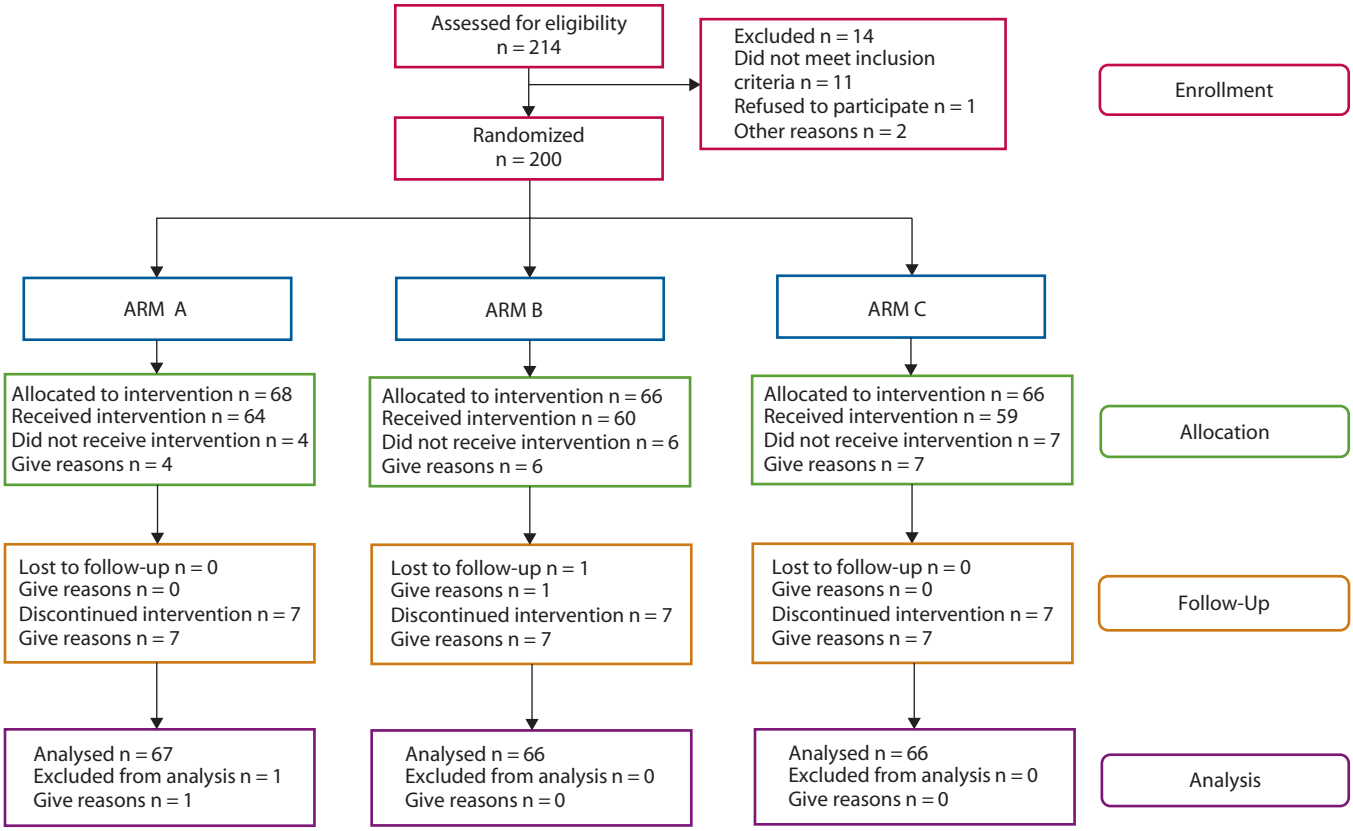

FIGURE 1. Consort 2010 flow diagram.

The incidence of fistula-in-ano ranges from 1.1 to 2.2 per 10,000 population per year.[1,2] Although most cases are easily treated by surgery, management of "complex fistulas" remains a challenge.[3–5] Limited surgery may result in recurrence, whereas aggressive surgery is associated with fecal incontinence.[3]

Application of autologous adipose-derived stem cells (ASCs; Cx401) represents a novel approach for enhancing regeneration and/or repair of damaged tissues[6,7] in an environment particularly unfavorable for wound healing.[8–11] It has been hypothesized that the immunoregulatory and anti-inflammatory properties of ASCs may work together to accelerate healing.[12,13] ASCs are purified from subcutaneous fat,[14,15] which can be readily and safely be obtained by liposuction.[11] The process yields 100 times more stem cells than bone marrow aspirates[16] and is considered safe.[17]

A previous proof-of-concept phase I clinical trial in fistulizing Crohn's disease[18] and a phase II clinical trial in fistulas of cryptoglandular origin and associated with Crohn´s disease[19,20] found ASCs to be safe and effective. The aim of the present phase III trial was to further investigate the effectiveness and confirm the safety of ASCs in the treatment of cryptoglandular complex fistula-in-ano.

MATERIAL AND METHODS

Once appropriate ethics committee and regulatory approval (Spanish Medicines Agency) had been granted, 214 consecutive patients with 1 solitary draining cryptoglandular complex fistula-in-ano from 19 centers in Spain were screened (Fig. 1). Patients were ascertained for any condition that might prevent the patient from remaining in the study until week 26. Patients of reproductive potential were required to use an acceptable form of contraception throughout the study and 6 months beyond.

Two hundred patients met the inclusion criteria (Fig. 2) and were enrolled. Informed consent was obtained. Fistula diagnosis was performed by physical examination and pelvic MRI in all patients. A fistula was considered to be complex if it presented at least one of the characteristics defined by the American Society of Colon and Rectal Surgeons.[4]

Protocol

This multicenter, single-blind, randomized, add-on clinical trial performed on 3 parallel groups was designed to evaluate the efficacy and safety of ASCs in the treatment of cryptoglandular complex fistula-in-ano. Patients were enrolled from March 2007 through August 2009. MRI ruled out collections >2 cm related to the fistulous

Inclusion criteria:
1. No identifiable fistula tract under the perianal skin and/or the fistula tract parallel to the rectum;
2. Fecal incontinence in transphincteric fistulas;
3. Risk factors for anal incontinence;
4. At least one previous operation due to fistulous disease (fistulectomy or advancement flap);
5. Suprasphincteric tracts shown by the MRI.
Seton presence was allowed until the therapy was applied.

Exclusion criteria:
1. Patients diagnosed with IBD, rectovaginal fistula, or acute sepsis;
2. Liposuction not technically possible;
3. Perianal surgery needed for reasons other than fistulas;
4. Presence of 2 or more perianal fistulas or collections >2 cm;
5. Allergy to local anesthetics or gadolinium;
6. Alcohol or other addictive substance abuse within last 6 months;
7. HIV, HBV, or HCV active or latent infection;
8. Mayor surgery within 28 days of recruitment;
9. Presence of a malignant tumor in the past 5 years;
10. Immunomodulatory treatment for reasons other than the fistula within 6 months;
11. A severe medical or psychiatric disease.

FIGURE 2. Inclusion and exclusion criteria. HBV = hepatitis B virus; HCV = hepatitis C virus.

tract. Patients with collections were excluded unless they resolved after surgical toilette. All enrolled patients completed a quality-of-life questionnaire (SF-36Q).[21] The Incontinence Wexner Score[22] (IWS) and the severity of the fistula were evaluated. We designed a "fistula complexity score" (FCS) that included 5 items related to fistula severity (Fig. 3).

Within 2 weeks of screening, patients were randomly assigned 1:1:1 to receive 1 of 3 treatments: 2×10^7 million ASCs (group A), 2×10^7 ASCs plus fibrin glue (group B), or fibrin glue and placebo (saline solution) (group C). Randomization was performed by an independent organization (Pivotal S.L., Madrid, Spain). An allocation list was generated by use of SAS (Statistical Analysis System version V9.2, SAS Institute Inc). A randomized block design, stratified by center and the presence/absence of collections at the baseline MRI, was created. The blocks were of size 6 with 3 treatments and 2 patients on each treatment.

Patients were blinded to the therapy administered, but the surgeons involved in patient´s treatment, follow-up, and general data collection were not. A surgeon without relationship to the center and without knowledge of the treatment administered ("blinded evaluator") evaluated fistula healing at weeks 12 and 24 to 26. This surgeon did not have access to the patients´ clinical records or communication with the investigator surgeon.

Within 4 weeks of the enrollment, all subjects underwent liposuction for production of the ASCs to be used during the study (group A and B) or at study completion to undergo ASCs implantation in the event that the primary hypothesis was confirmed (group C). The liposuction was performed by qualified plastic surgeons under local anesthesia (1% mepivacaine), and 100 mL of fat were obtained. Within 24 hours, the material was sent in 50-mL tubes stored at 2 to 8°C to Cellerix S.A., where the samples were processed as described previously.[18,19]

One week before the implantation, cells were prepared at a concentration of 1×10^7 cells/mL and placed in 2-mL vials. Samples from each vial were tested before release as described elsewere.[18,19]

Fibrin glue (Baxter Inc, Spain) is a biological sealant containing human fibrinogen, bovine aprotinin, and human thrombin. The recommended dose is 1 mL per 4-cm^2 surface area; 1 to 5 mL were used.

Treatments were administered in an operating room, under a standard day-surgery protocol, as described in Figure 4.

Follow-up

Fistula healing, IWS, FCS, and SF-36Q were assessed at 1, 4, and 12 weeks. A second MRI was performed at 12 weeks. If the fistula remained open, a second double dose of ASCs

External opening:
- One (1)
- Two (2)
- More (3)

Internal opening:
- Yes (1)
- No (2)

Fistula tract type:
- Subcutaneous (0)
- Transsphincteric (1)
- Suprasphincteric (3)
- Horseshoe (4)

Cavities size:
- No (0)
- Small (2)
- Big (3)

Suppuration present:
- No (1)
- Yes (3)

FIGURE 3. Complexity Fistula Score (points). The resulting score ranged from 3 to 15, with higher scores indicating greater complexity.

Group A:
1. Characterization of tract and internal opening;
2. Tract curettage;
3. Closure of the internal opening, if possible, with a Vicryl 2/0 (Ethicon, Spain) stitch.
4. Resuspension of cells by gentle manual shaking of vial and immediate application of the therapy to prevent cells from settling.
5. Injection of the suspension of cells through a long fine needle (Abocatt 20; Terumo Corporation, Belgium) into the tract walls, with half the cells placed in the internal opening and intersphincteric tracts and the other half in the tract walls. Injections were no deeper than 2 mm.

Group B:
1-5 As described above.
6. Sealing of the fistulous tract with fibrin adhesive.

Group C:
1-3 As described above
4. Injection of matching for ASCs (saline solution, 5 mL) through a long fine needle (Abocatt 20; Terumo Corporation, Belgium) into the tract walls, with half the placebo being placed in the internal opening and intersphincteric tracts and the other half in the tract walls. Injections were no deeper than 2 mm.
5. Sealing of the fistulous tract with fibrin adhesive.

FIGURE 4. Surgical protocol. ASC = adipose-derived stem cells.

(4×10^7 million), double dose of ASCs plus fibrin glue, or fibrin glue plus placebo was administered, and the patient was reassessed according to the same protocol. The second dose was administered within 2 weeks of week 12. The primary end point (fistula healing) was evaluated 24 weeks after the first dose was administered (26 weeks if a second dose was required).

Evaluation of Efficacy

The "blinded evaluator" assessed healing at week 12 and 24 to 26. Healing was defined as absence of drainage through the external openings, complete reepithelization of external openings, and the absence of collections >2 cm by MRI.

The primary efficacy end point was defined as the proportion of patients whose fistula healed at 24 to 26 weeks according to the "blinded evaluator."

In secondary analyses, the proportion of patients treated with ASCs with improved quality of life, IWS, and severity of the fistula at 24 to 26 weeks were evaluated.

An additional analysis regarding seton or antibiotic use, fistula type according to Parks Classification,[5] and center where the patient was treated was performed "post hoc."

Evaluation of Safety

Safety was assessed by the incidence of adverse events (AEs) and serious adverse events (SAEs). Patients were monitored for AEs at each study visit. Special attention was given to any signs of abnormal tissue formation and persistent or increased inflammation.

Statistical Analysis

The primary analysis was performed by intention-to-treat (ITT). The ITT population for efficacy was defined as all randomly assigned patients who received at least 1 dose of the corresponding treatment and returned for at least 1 clinical evaluation. The ITT population for safety was defined as all randomly assigned patients. All tests were 2-sided. Significance was set at 5%.

The analysis of efficacy was performed by comparing the absolute difference in proportions in groups A and B (active treatment) and C (placebo) with a χ^2 test. The relative risk for healing with was calculated along with 95% CIs. An ANOVA was used for group comparisons for the SF-36Q score, with the use of baseline results as the covariate. The difference between means for the 2 groups was used, and the 95% CI was calculated.

The incidence of AEs and SAEs was analyzed by comparing the absolute differences in proportions in groups A and B and C (median and 95% CI) with a χ^2 test.

For the sample-size calculation, the primary hypothesis was evaluated by using a statistical significance test between the 3 groups. For a 30% difference between the rate of patients who would achieve closure of fistulas treated with ASCs in comparison with the control group, a sample size of 177 evaluable patients would have been needed to detect this difference with 90% power ($\alpha = 0.05$; 2-tailed). Assuming 15% losses to follow-up, 30 additional patients were included.

Kruskal-Wallis test was used to compare differences in the IWS. Wilcoxon Mann-Whitney and χ^2 test was used to compare the baseline risk factors values for patients from

Hospital La Paz vs patients from other centers. Logistic regression models were used to warrant reporting results in specific centers and to test the influence of imbalance in baseline features in different groups.

Long-term Evaluation

In order to assess AEs and the maintenance of the fistula closure, patients were evaluated by a "blinded evaluator" 48 weeks (12 months) after the therapy was administered. This was designed as an independent study, and patients signed a new consent form to be enrolled. A physical examination was performed, and IWS, FCS, SF-36Q, and AE were assessed. A new MRI was performed. Although 148 patients signed the consent form, only 135 underwent a new MRI and were finally included.

RESULTS

All results provided are as per "blinded evaluator" and were similar to the investigator assessment.

The ITT population comprised 183 patients of the 200 enrolled (Fig. 5). Of these, 165 (90.2%) completed the study. Reasons for withdrawal are described in Table 1. Demographic and clinical characteristics at baseline are shown in Table 2.

As shown in Table 3, the fistula healing rates at week 12 were 26.56%, 38.33%, and 15.25% in arms A, B, and C ($p = 0.01$). A total of 61.75% (n = 113) patients received 2 doses of treatment. The fistula healing after the second dose was 39.1% (n = 25), 43.30% (n = 26), and 37.29% (n = 22) ($p = 0.79$). Time to healing was similar in all groups. The proportion of patients with a healed fistula at week 12 who experienced reopening of the fistula at week 24 to 26 was 25.00%, 14.29%, and 11.11% ($p > 0.5$). Those patients did not receive a second dose of treatment.

Additional analysis regarding seton or antibiotic use and fistula type did not show statistically significant differences (Table 4).

We studied the influence of the institution in which the patients were treated and statistically significant differences between "La Paz" University Hospital (LPUH, the center that pioneered this therapy) and the other participating centers (OCs) were found. Healing rates at LPUH were 54.55%, 83.33%, and 18.18% in arms A, B and C ($p = 0.007$), whereas the OCs combined reported fistula closure in 35.8%, 33.3%, and 42.6% ($p = 0.40$). An additional analysis was performed defining these 2 groups, and an imbalance in the baseline features of the fistulas was found (Table 5). Figure 6 shows healing rates by center. A logistic regression model warranted the report of results of a specific center (LPUH) with $p = 0.0197$. For LPUH, the treatment is a significant effect for the fistula healing ($p = 0.025$), with best results if the treatment is "ASCs + fibrin glue" (OR 19.99; 95% Wald confidence limits 2.28–175) with an area under the curve (AUC) = 0.7815. In OCs, treatment was not a statistically significant effect to explain fistula healing. A multivariate logistic model was also built to explain fistula healing as a function of the treatment arm, center, IWS, presence of seton, fistulous tract above sphincter, FCS, anal incontinence, and perianal abscess at baseline. The results indicated that "center" and "FCS" were the significant factors ($p = 0.024$). This indicates that FCS is a risk factor (OR 0.75; 95% Wald confidence limits 0.61–0.91), and there is a higher probability of fistula closure at LPUH than at OCs (OR 2.36; 95% Wald confidence limits 1.13–6.11) with an AUC = 0.6466. Stratified analysis showed that at HULP the only statistically significant effect is the FCS. No statistically significant effect was found for OCs.

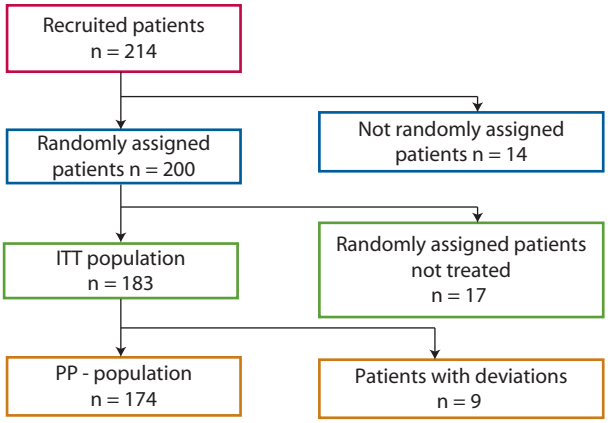

FIGURE 5. Recruitment and random assignment of study patients. Intent-to-treat (ITT) population = all randomly assigned patients who met the main inclusion and exclusion criteria and had received at least 1 dose of treatment; Per-protocol (PP) population = patients in the ITT population who showed no major protocol deviations. One patient (18001) was randomly assigned but did not receive treatment, and it was not possible to collect any additional information. As a result that patient was not recorded in the study database.

No statistically significant differences were found when comparing SF-36Q (mental and physical health) at inclusion and at 24 to 26 weeks. There was a reduction in

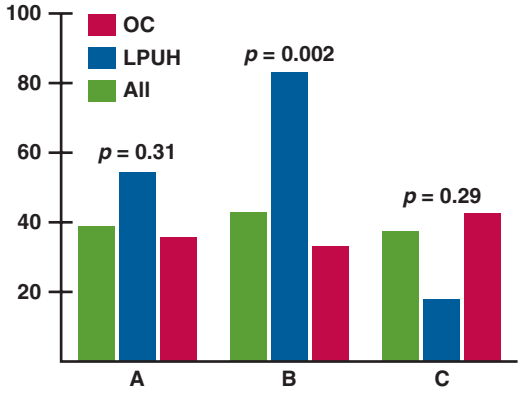

FIGURE 6. Efficacy at 24 weeks when divided by center. p values correspond to the χ^2 test to measure the association between grouped center and efficacy rate at 24/26 weeks (ie, efficacy rate LPUH vs OC). LPUH = La Paz University Hospital; OC = other centers.

TABLE 1. Reasons for patient withdrawal

	Randomization group			
Reasons for patient withdrawal	*A (n = 64)*	*B (n = 60)*	*C (n = 59)*	*Total*
Investigator's decision, n (%)	1 (1.56)	1 (1.66)	1 (1.69)	3 (1.64)
Adverse event, n (%)	0 (0.00)	0 (0.00)	4 (6.78)	4 (2.19)
Loss to follow-up, n (%)	0 (0.00)	1 (1.67)	0 (0.00)	1 (0.55)
Withdrawal of informed consent, n (%)	0 (0.00)	2 (3.33)	0 (0.00)	2 (1.09)
Other, n (%)	5 (7.81)	1 (1.67)	2 (3.39)	8 (4.37)
Total, n (%)	6 (9.38)	5 (8.33)	7 (11.86)	18 (9.84)

TABLE 2. Demographic and clinical characteristics at baseline (ITT population)

Variable	*A (n = 64)*	*B (n = 60)*	*C (n = 59)*	*p*[a]
Age, y, mean (SD)	49.78 (11.39)	47.27 (12.27)	50.85 (12.51)	0.25
Sex				
Male, n (%)	47 (73.44)	36 (60.00)	44 (74.58)	0.15
Race				
White, n (%)	64 (100)	60 (100)	58 (98.31)	0.32
Other, n (%)	0 (0)	0 (0)	1 (1.69)	
Disease factors				
Complex fistula at baseline, n (%)	64 (100.00)	60 (100.00)	59 (100.00)	–
Complexity of fistula score, mean (SD)	6.83 (1.80)	6.86 (1.82)	7.03 (1.89)	0.87
Anal incontinence, n (%)	7 (10.94)	13 (21.67)	14 (23.73)	0.14
Fistulous tract above sphincter, n (%)	27 (42.19)	28 (46.67)	26 (44.07)	0.88
Seton, n (%)	27 (42.19)	28 (46.67)	26 (44.07)	0.88
Incontinence Wexner score, mean (SD)	0.58 (2.04)	1.67 (3.31)	1.33 (2.90)	0.07
History of perianal abscess, n (%)	60 (93.75)	56 (93.33)	56 (94.92)	1.00
Previous perianal surgery, n (%)	58 (90.63)	54 (90.00)	51 (86.44)	0.72

ITT = intention-to-treat population

[a]p values corresponding to the comparison between treatment groups. For categorical variables the $\chi 2$ test or the Fisher exact probability test were applied. For continuous variables, a 1-way ANOVA model was applied, if the normality assumption was satisfied, or a nonparametric Kruskal-Wallis test in another case.

TABLE 3. Clinical fistula closure at weeks 12 and 24 (26) (ITT population)

	Randomization arm				
	A	*B*	*C*	*Total*	*p*
Clinical fistula closure at week 12 (ITT, investigator assessment), n (%)	20 (31.25)	22 (36.67)	10 (16.95)	52 (28.42)	0.04
Clinical fistula closure at week 12 (ITT, blind evaluator assessment), n (%)	17 (26.56)	23 (38.33)	9 (15.25)	49 (26.78)	0.01
Clinical fistula closure at weeks 24/26 (ITT, investigator assessment), n (%)	27 (42.19)	28 (46.67)	22 (37.29)	77 (42.08)	0.58
Clinical fistula closure at weeks 24/26 (ITT, blind evaluator assessment), n (%)	27 (42.19)	24 (40.00)	23 (38.98)	74 (40.44)	0.93

ITT = intention-to-treat population.

TABLE 4. Possible factors affecting healing

	Healing-randomization arm			
	A (n = 25)	*B (n = 26)*	*C (n = 22)*	*p*
Seton presence	10 (40.00)	13 (50.00)	11 (50.00)	0.71
Antibiotics use	21 (84.00)	26 (100.00)	19 (86.36)	0.09
Intersphincteric fistulas	1 (4.00)	2 (7.69)	1 (4.55)	1.00
Transsphincteric fistulas	20 (80.00)	17 (65.36)	20 (90.91)	0.09
Suprasphincteric fistulas	3 (12.00)	7 (26.92)	1 (4.55)	0.09

IWS in group A (0.86 ± 2.71 to 0.64 ± 2.52) and in group B (1.55 ± 3·43 to 0.95 ± 2·84), whereas the score increased in group C (1.09 ± 2.48 to 1.48 ± 3·11) ($p = 0.09$).

In all 3 treatment groups, mean FCS reduced from the inclusion to the end of the study, but this difference was not statistically significant.

Most patients had at least 1 AE (59 (90.8%), 49 (84.5%), and 51 (85.0%)) in groups A, B, and C ($p = 0.51$). Most of these AEs were nonserious. There were 17 different AEs reported in more than 5% of the cases. The most frequent AEs were proctalgia (80 (43.7%)), abscess drainage (41 (22.4%)), pain (25 (13.7%)), perianal abscess (24 (13.1%)), pyrexia (17 (9.3%)), swelling (12 (6.6%)), and pruritus (12 (6.6%)). There were no statistically significant differences within groups. AEs were common but mostly minor.

There were 37 SAEs in 30 patients. All but 4 SAEs were unrelated to study treatment (Table 6). There were 3 SAEs related to study procedures but not with the therapy itself (excluding liposuction). There were no deaths during the study.

Long-term Results

All results provided are as per "blinded evaluator" assessment. Of the 148 patients who were enrolled in the long-term study, 135 had an MRI evaluation at 12 months. The demographic and clinical features are presented in Table 7.

Fistula closure was reported in 57.1%, 52.4%, and 37.3% in arms A, B, and C ($p = 0.13$). Table 8 compares healing at 24 to 26 weeks and at 48 weeks. When the use of cells with or without fibrin (groups A + B) was compared with fibrin alone (group C), the healing rate of patients treated with ASCs was 2 times higher ($p = 0.048$, relative risk 2.039, 95% IC 1–4.15).

Of the patients initially enrolled (n = 148), 1 patient presented an AE related to ASC therapy (0.7%) and none related to fibrin adhesive. There were no related SAEs.

Figure 7 summarizes fistula healing per group over time.

DISCUSSION

This trial was conceived and designed following the encouraging results of 2 previous trials, one in phase I and one in phase II, for the treatment of complex fistula-in-ano.[18,19,23] A healing rate of 71% with almost no risk of incontinence and a low recurrence rate (12%) were achieved in the phase II trial with 49 patients.[19] The phase III trial was conducted to evaluate the efficacy and safety of ASCs in 200 adult patients with cryptoglandular complex fistula-in-ano.

All results discussed are as per "blinded evaluator" assessment. The outcomes at 12 weeks after therapy administration (healing rates, 26.56%, 38.33%, and 15.25%; p = 0.01) were comparable to previous studies,[18,19] and significantly higher healing rates were reported in the groups receiving ASCs versus fibrin glue alone. At 24 to 26 weeks (after a second dose if applied), the healing rates were still good (39.01%, 43.3%, and 37.3%), but there

TABLE 5. Diseases factors at baseline grouped by center (ITT population)

	Randomization arm						
	A		*B*		*C*		
	LPUH (n = 11)	*OCs (n =5 3)*	*LPUH (n = 12)*	*OCs (n = 48)*	*LPUH (n = 10)*	*OCs (n = 49)*	*p*[a]
Wexner score, mean (SD)	0.64 (0.92)	0.57 (2.21)	3.33 (3.77)	1.25 (3.09)	1.50 (1.84)	1.29 (3.09)	<0.0001[b]
Fistulous tract above sphincter, n (%)	8 (72.73)	19 (35.85)	8 (66.67)	20 (41.67)	8(80.00)	18 (36.73)	0.0003[c]
Complexity score at baseline, mean (SD)	8.00 (2·61)	6.58 (1.51)	6.91 (1.92)	6.85 (1.81)	8.40 (1.84)	6.75 (1.79)	0.004[b]
Presence of seton, n (%)	0 (0.00)	27 (50.94)	6 (50.00)	22 (45.83)	1 (10.00)	25 (51.02)	0.003[c]
Anal incontinence, n (%)	4 (36.36)	3 (5.66)	7 (58.33)	6 (12.50)	5 (50.00)	9 (18.37)	0.0001[c]

ITT = intention-to-treat population; LPUH = "La Paz" University Hospital; OCs = other centers.
[a] *p* value corresponding to the statistical test applied to analyze the center effect.
[b] *p* value corresponds to the nonparametric Wilcoxon Mann-Whitney *U* test applied to compare the baseline risk factor values for patients from LPUH vs patients from OCs (continuous variables).
[c] *p* value corresponds to the Cochran-Mantel-Haenszel test applied to compare the baseline risk factor values for patients from LPUH vs patients from OCs (categorical variables)

TABLE 6. Related serious adverse events

Treatment arm	*Age, sex*	*SAE*	*Outcome*	*Intensity*	*Causality*
C	39, male	Perianal abscess	Resolved	Mild	Related
C	69, male	Perianal abscess	Resolved	Severe	Related
B	32, female	Pyrexia	Resolved	Mild	Related
B	63, female	Injection site nodule	Resolved	Mild	Related

SAE = serious adverse event.

were no statistically significant differences between groups (p = 0.79). Time to healing was also similar in all groups. Given that the risk of anal sphincter injury is almost negligible, these healing rates were still encouraging, but because healing at 24 to 26 weeks was the primary outcome measure, the higher efficacy of ASCs in comparison with the control treatment was not established. In view of this unexpected result, we searched for factors that might explain why fistula closure rates were lower than in the previous studies.[18,19] Seton placement, antibiotic use, and type of fistula were evaluated, but statistically significant differences between groups were not found. With further analysis warranted by logistic regression analysis, patients were then analyzed according to those treated at LPUH, the pioneer center where this therapy was first developed, and the OCs that were performing this therapy for the first time. Healing rates at LPUH were 54.55% and 83.33% for groups A and B, whereas group C achieved a rate of 18.18% (p = 0.007). However, healing rates in the OCs were 35.8%, 33.3%, and 42.6% (p = 0.40). These findings were not consistent with previous studies, so we reanalyzed the population in search of significant baseline differences between LPUH and the OC. We found that IWS,[22] anal incontinence grade, FCS, and seton placement were different in both groups (Table 6). Regression analysis models found "center of treatment" and "FCS" as the significant factors determining fistula healing. A picture emerges of a selection for more complex fistulas in patients treated at LPUH, perhaps because the American Society of Colon and Rectal Surgeons recommendations,[4] on which the inclusion criteria were based, are not sensitive enough to discriminate the more complex fistula, and simpler cases were selected in OCs, increasing the likelihood of healing in the fibrin-alone group. We believe fibrin glue application could be an acceptable initial therapy to manage selected patients. The type of fistula that should be treated with fibrin glue has been defined as long (4 cm), simple, nonramificated fistula.[24] Long-term poor results (16%) have been reported when treating complex fistula-in-ano,[25] and they are consistent with the LPUH poor healing rate. This does not explain, however, why LPUH achieved excellent results when very complex fistulas-in-ano were treated with ASCs, and healing rates were much lower in OCs. Unfortunately, prospective data and analyses are not available to help answer this question. A technical questionnaire, which did not form part of the study, was distributed to evaluate surgical skills and preferences of surgeons. Interestingly, fistula curettage was very aggressive in some hospitals, with the use of a curettage device, and was less aggressive in others, in which it was performed with gauze. A few centers used hydrogen peroxide to look for the internal opening, instead of saline solution, to avoid cell damage. These are all factors that might influence the final outcome.

TABLE 7. Long-term trial demographic and clinical characteristics at baseline

Variable	*A (n = 49)*	*B (n = 46)*	*C (n = 53)*
Age, y, mean (SD)	50.84 (11·71)	46.91 (12.29)	50.98 (12.55)
Sex, male, n (%)	36 (73.5)	25 (54.3)	41 (77.4)
Weight, kg, mean (SD)	81.38 (13·98)	76.19(18.04)	81.62 (14.35)
Fistula type, n (%)			
Intersphincteric	0 (0.0)	2 (4.3)	1 (1.9)
Transsphincteric	38 (77.6)	34 (73.9)	40 (75.5)
Suprasphincteric	11 (22.4)	10 (21.7)	11 (20.8)
Extrasphincteric	0 (0.0)	0 (0.0)	1 (1.9)
			p > 0.5

Another possibility is that the greater experience of the team at LPUH had influence on the outcomes. The team has been performing ASC implantations to treat fistula-in-ano since 2002 in more than 150 procedures. The most procedures performed by another center was 24, and 11 centers performed procedures on fewer than 10 patients. Objective evidence of a learning effect is hard to obtain. We note, however, that in the phase II study, the healing rate was of 70% (excluding patients with Crohn's disease), whereas LPUH achieved a healing rate of 83.3%, although the baseline characteristics of the patients were similar.

Is this approach to treat complex fistula-in ano worth pursuing? The outcome of this phase III trial was negative in that the primary outcome measure was not met, but there are certain indications, in line with the preceding studies, suggesting that the procedure can be effective in the right conditions. Furthermore, long-term results showed that the healing rate at 1 year was double for the use of ASCs with or without fibrin in comparison with fibrin glue alone, but this should be interpreted cautiously, because a 27% loss of sample size was encountered in comparison with the main study. Any decision will be influenced by the fact that surgery is the only accepted effective treatment for complex fistula-in-ano. Despite healing rates above 60%, surgery is associated with incontinence rates between 10% and 35%[26–29] and recurrence rates between 11% and 45%.[26–29] With ASC therapy, there is no injury to the anal sphincter because tract resection is not required and repeated doses can be used to increase the chance of healing.

TABLE 8. Fistula closure at 24 to 26 weeks and 48 weeks, excluding patients without MRI

Variable	*A (n = 42)*	*B (n = 42)*	*C (n = 51)*	*Total (n = 135)*	*p*[a]	*P* A vs B	*P* A vs C	*P* B vs C
Healing 24–26 wk, n (%)	20 (47.6)	23 (54.8)	18 (35.3)	61 (45.2)	0.16	1.00	0.05	0.05
Evaluation 48 wk, n (%)	24 (57.1)	22 (52.4)	19 (37.3)	65 (48.1)	0.13	0.66	0.05	0.14
Relapse, n (%)	0 (0.0)	4 (9.5)	0 (0.0)	4 (3.0)	0.010	0.11	–	0.03

[a]Overall p value; assessment provided by the "blinded evaluator."

CONCLUSION

ASC therapy achieved moderate healing rates without risk of anal incontinence or procedure-related AEs; however, healing rates were not superior to fibrin adhesive at 24 to 26 weeks (primary end point). Further studies should be performed to better define the most beneficial scenario for ASC therapy. To our knowledge, this is the first report of a randomized controlled phase III trial in which ASCs were used to treat a medical condition.

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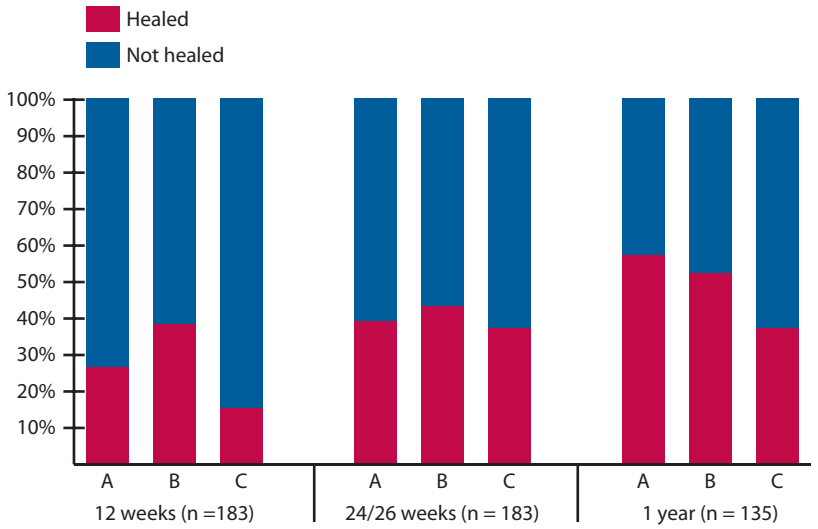

FIGURE 7. Fistula healing per group over time.

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