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HUMAN CLINICAL ARTICLE

Treatment of Crohn's-Related Rectovaginal Fistula With Allogeneic Expanded-Adipose Derived Stem Cells: A Phase I–IIa Clinical Trial

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ABSTRACT

The aim of this clinical trial was to determine the safety and feasibility of expanded allogeneic adipose-derived stem cells to treat Crohn's-related rectovaginal fistula (CRRVF). We designed a phase I–II clinical trial (https://ClinicalTrials.gov, NCT00999115) to treat 10 patients with CRRVF. Patients receiving biological therapy during follow-up were excluded. Curettage was performed, and a vaginal or rectal flap was added if the surgeon considered it necessary. The therapeutic protocol included intralesional injection of 20 million stem cells in the vaginal walls (submucosal area) and fistula tract. Healing was evaluated 12 weeks later. If the fistula had not healed, a second dose of 40 million stem cells was administered. Patient follow-up was 52 weeks from last cell injection. Healing was defined as re-epithelialization of both vaginal and rectal sides and absence of vaginal drainage. Cytokines and immunological blood tests were monitored. Serious adverse events or rejection issues were not observed. Five patients were excluded because biologic drugs were required to treat a Crohn's disease flare-up during follow-up. Cytokine profiles and immunotoxicity assays showed no statistically significant alterations. Sixty percent of the nonexcluded patients achieved a complete healing. Expanded allogeneic adipose-derived stem-cell injection is a safe and feasible therapy for treating CRRVF, and the healing success rate seems promising (60%). The results of this trial encourage further exploration into this therapy. STEM CELLS TRANSLATIONAL MEDICINE *2016;5:1441–1446*

SIGNIFICANCE

This may be the first publication in which allogeneic stem cells to treat rectovaginal fistula in Crohn´s disease seem to be a feasible and safe treatment. Additional studies are necessary to confirm the efficacy profile of the allogeneic stem cells strategy in a controlled design.

INTRODUCTION

Crohn's-related rectovaginal fistula (CRRVF) treatment continues to pose a challenge [1]. Although several different therapeutic approaches have been described, their success rates vary. When surgical treatments are indicated, no single technique produces a clearly superior outcome for this condition. Limited surgical approaches may result in recurrence, whereas aggressive surgery is associated with fecal incontinence [2].

Application of adipose-derived stem cells (ASCs) is a novel approach for enhancing regeneration or repair of damaged tissues [3, 4] in an environment as particularly unfavorable for wound healing as CRRVF [5–8]. It has been hypothesized that the therapeutic effect of ASCs may be due to their immunoregulatory and anti-inflammatory properties, which may work together to accelerate healing [9, 10]. ASCs are isolated from subcutaneous fat [11, 12], which can be readily and safely obtained by liposuction [8]. The process yields 100 times more stem cells than do bone marrow aspirates [13] and is considered safe [14].

Previously, our group performed a proof-of-concept study, a phase I clinical trial of fistulizing Crohn's disease [15], a multicenter phase II clinical trial of cryptoglandular and Crohn's-related fistulas [16, 17], and a phase III trial studying complex anal fistulas. These studies found autologous ASCs to be safe and effective [18]. In the present study, we investigate the safety and efficacy of cells from a healthy donor, a method that would reduce the inherent costs and time-consuming processing of autologous ASCs. In the present trial we used expanded allogeneic adipose-derived stem cells (e-ASC) obtained from a healthy donor to treat

Table 1. Patient demographics and fistula characteristics

Patient	Age (years)	Years of evolution of CD	CRRVF previous surgeries	Previous medical treatment	No. of tracts	Diameter (mm)
ALOREVA 01	34	4	2	Azathioprine	1	5
ALOREVA 02	32	5	3	Azathioprine	2	2
ALOREVA 03	52	10	1		1	20
ALOREVA 04	34	19	2		1	20
ALOREVA 05	45	10	4		1	3
ALOREVA 06	36	17	1		1	2
ALOREVA 07	31	16	1	Azathioprine	3	2
ALOREVA 08	49	23	2	Azathioprine	1	3
ALOREVA 10	55	1	1	Mesalazine	1	4
ALOREVA 11	34	11	1	Adalimumab	1	3
				steroids		
Mean	35	11.6	1.8		1.3	4.8

Abbreviations: ALOREVA, ALOREVA clinical trial (Allogeneic Stem Cells Derived From Lipoaspirates for the Treatment of Rectovaginal Fistulas Associated to Crohn's Disease); CD, Crohn's disease; CRRVF, Crohn's-related rectovaginal fistula.

CRRVF. Only one surgery was performed on each patient to inject the cell after expansion.

Patients and Methods

Protocol was designed to evaluate the safety and feasibility of e-ASCs for treatment of rectovaginal fistula due to Crohn's disease. The Ethical Committee of La Paz University Hospital (Madrid, Spain) and the Spanish Agency of Medicines (AEMPS) according to current legislation, approved the trial. This study is performed according to the amended Declaration of Helsinki (https://ClinicalTrials.gov, Identifier NCT00999115)

Eleven women were enrolled in the trial between October 2009 and June 2010; demographics and the fistula features appear in Table 1. Inclusion criteria were as follows: age more than 18 years; Crohn's disease diagnosis (based on clinical, endoscopic, radiologic, and pathological criteria, with tests performed at least 1 year before inclusion in this study); presence of a rectovaginal fistula; and one of the following: (a) at least one previous surgery for the rectovaginal fistula; (b) physical conditions that excluded the possibility of undergoing liposuction. The most relevant exclusion criteria were severe proctitis or endoluminal disease; Crohn's Disease Activity Index $\geq$ 201; perianal sepsis; anti-tumor necrosis factor (TNF) administration in the 8 weeks previous to enrollment; and tacrolimus or cyclosporine administration in the 4 weeks prior to cell application.

Eleven patients were enrolled, and 10 patients met the inclusion and exclusion criteria. e-ASCs were obtained from a healthy anonymous donor through liposuction. Fat was processed and manufactured by Cellerix S.L. (Madrid, Spain, http://www.pharmatech.es/empresas/cellerix-sl)/TiGenix S.A.U. (Leuven, Belgium, http://www.tigenix.com), with permission from Spanish regulatory authorities to obtain e-ASCs [15, 16, 18].

Cell Characterization

The e-ASCs were characterized according to the Guideline on Cell-Based Medicinal Products (EMEA/CHMP4/410869/2006) and the Reflection Paper on Stem Cells (EMA/CAT/571134/2009) (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/02/WC500101692.pdf). The phenotype of mesenchymal stem cells is according to the International Federation for Adipose Therapeutics and Science and the International Society for Cellular Therapy [19].

Study Endpoints

The primary endpoint of the present trial was to analyze the safety and feasibility of e-ASCs to treat CRRVF. The secondary endpoint was to monitor the patient cytokine levels and immunological test results to assess any rejection issues due to e-ASCs. The tertiary endpoint was to obtain preliminary efficacy data necessary for a subsequent phase II–III trial design.

Treatment Procedure and Follow-Up

The trial protocol is summarized in Figure 1 and was as follows: (a) the vaginal and rectal openings were identified, as was the fistula tract or tracts; (b) a curettage was performed and a vaginal or rectal flap was added if the surgeon considered it to be necessary; (c) cells (20 million e-ASCs) were injected into the vaginal opening and fistula tract in the submucosal area.

The patients were discharged between 1 and 4 days postoperatively, and follow-up visits to the outpatient clinic were scheduled for1, 4, 8, 12, 24, and 52 weeks after cell administration. Quality of life score (QoL score), 36-Item Short-Form (SF-36) Health Survey [20], and fecal incontinence severity index [21] were assessed before the surgery and at each follow-up visit. Those patients who did not achieve total re-epithelialization of the fistula tract at the 12-week time point were rescued with a second cell dose (40 million e-ASCs). A tertiary endpoint defined as healing of the CRRVF was evaluated at 52 weeks of follow-up (1 year). A CRRVF was considered healed when the vaginal and rectal walls showed complete re-epithelialization and absence of vaginal drainage, including feces, flatus, or suppuration.

Evaluation of Safety and Follow-Up of Cytokine Levels

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

Prior to the surgery and at each follow-up visit, cytokine levels were measured by enzyme-linked immunosorbent assay (ELISA),

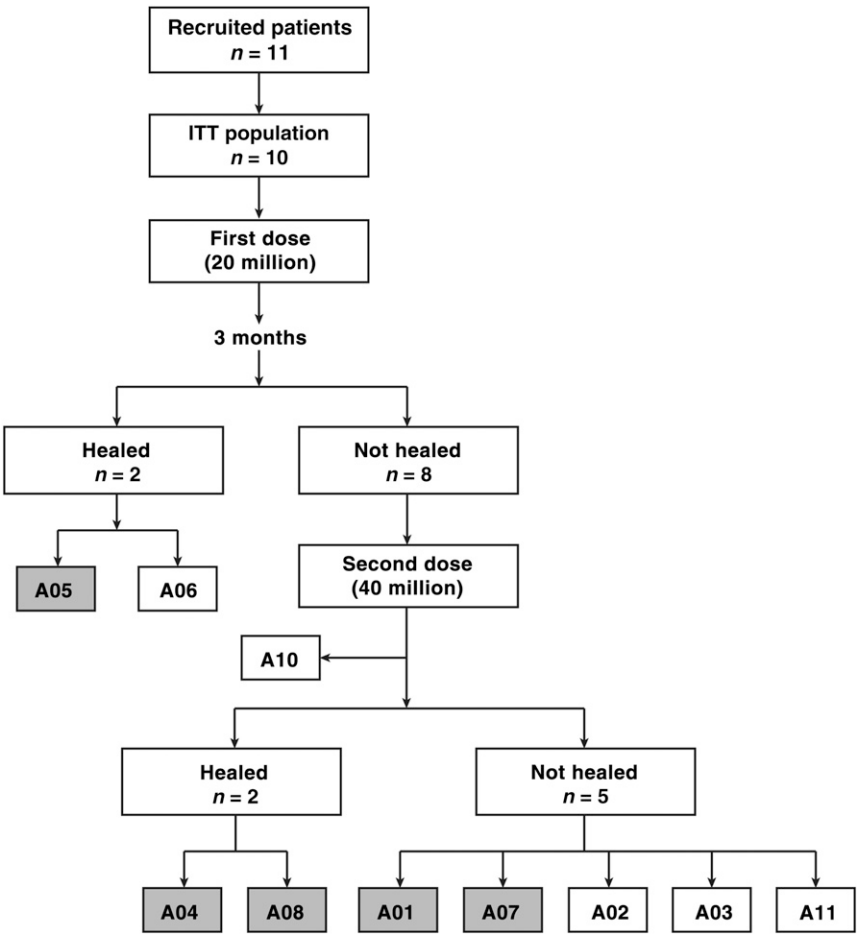

Figure 1. Flowchart and results. The gray boxes indicate patients who completed the trial; the white boxes indicate patients who were excluded and did not complete the trial. The patients were excluded because of severe flare-up or biological medication needed. HEALING, fistula closure at 1 year of follow-up; ITT, intention to treat.

although a serum test performed by an external Good Manufacturing Practices laboratory (Balagué Center, Barcelona, Spain, http://www.pca3.org/pro/labs/spain/balagu%C3%A9-center). The cytokines determined were TNF-α, interleukin (IL)-10, IL-6, IL-2, transforming growth factor-β, interferon γ, and IL-12.

Prior to the surgery, on the day of implantation, and 24 hours after surgery, immunological tests were performed by using blood obtained through a blood test by TiGenix S.A. (Tres Cantos, Spain), testing for immunoglobulin IgG and IgM.

Statistical Analysis

Although the sample size was set without testing for statistical power considerations, the number of 10 evaluable patients was considered to be sufficient to fulfill the aim of the study, at least after the first dose. The primary outcome measure was the incidence of treatment-related AEs (biosafety); this was assessed by calculating the proportion of subjects with at least one AE associated to treatment, along with corresponding binomial exact 95% confidence intervals.

Because of the low number of patients included, no statistical tests were performed; instead, we present our results as general figures or simple percentages. Data regarding cytokine levels were analyzed with the Wilcoxon test.

Results

Primary and Secondary Outcome Measures

No SAEs or mild AEs were found to be related to the e-ASC applications. The cases of Crohn's disease flare-up were considered to be the result of poor disease control and were not deemed to be related to stem-cell therapy. Finally, no statistically significant differences were found when cytokine blood levels were measured, and none of the patients showed changes in levels of IgG or IgM analyzed by ELISA. Therefore, the same donor could be used in subsequent administrations. (Table 2). As a consequence of our results, we concluded that e-ASCs can be considered safe. QoL score, SF-36 score, and fecal incontinence severity score did not show any significant differences before and after the treatment (data not shown).

Third Outcome Measure

Healing data at 52 weeks are summarized in Figure 1. Eleven patients were enrolled in the trial, and 10 of them received a dose of 20 million e-ASCs. Twelve weeks after the first injection, two fistulas had healed, and eight remained open. One of the two patients whose fistula closed after the first dose and another patient with an open fistula did not complete the study because

Table 2. Cytokine and immunological tests

Patient A[a]	Visit	IL-1B pg/ml RV: <8	IL-2 U/ml RV: <1.2	IL-6 pg/ml RV: <14	IL-10 pg/ml RV: <19	IL-12 + p40 pg/ml RV: 24-369	TNF-α pg/ml RV: <16	IFN-γ U/ml RV: <0.8	Patient B[b]	A	B	C
ALOREVA 01	0	<3.9	<1.20	<1.56	<19	110	<16	<0.50	ALOREVA 01	Negative	Negative	Negative
ALOREVA 01	3′	<3.9	<1.20	<1.56	20	85.6	<7.8	<0.50				
ALOREVA 02	0	<3.9	<1.20	5.53	<19	231	<7.8	<0.50	ALOREVA 02	Negative	Negative	Negative
ALOREVA 02	3′	<3.9	<1.20	<1.56	<19	89.1	<7.8	<0.50				
ALOREVA 03	0	<3.9	<1.20	5.38	<19	270	<7.8	<0.50	ALOREVA 03	Negative	Negative	NS
ALOREVA 03	3′	<3.9	<1.20	2.91	<19	140	<7.8	<0.50				
ALOREVA 04	0	<3.9	<1.20	<1.56	<19	196	<7.8	<0.50	ALOREVA 04	Negative	Negative	Negative
ALOREVA 04	3′	31	<1.20	<1.56	<19	233	<7.8	<0.50				
ALOREVA 05	0	<3.9	<1.20	6.80	<19	179	<7.8	<0.50	ALOREVA 05	Negative	Negative	NS
ALOREVA 05	3	<3.9	<1.20	4.02	<19	<17	<7.8	<0.50				
ALOREVA 06	0	<3.9	<1.20	4.98	<19	269	<7.8	<0.50	ALOREVA 06	Negative	Negative	NS
ALOREVA 06	3	<3.9	<1.20	6.46	<19	217	<7.8	<0.50				
ALOREVA 07	0	<3.9	<1.20	7.06	<19	153	<7.8	<0.50	ALOREVA 07	Negative	Negative	Negative
ALOREVA 07	3′	<3.9	<1.20	<1.56	<19	209	<7.8	<0.50				
ALOREVA 08	0	<3.9	<1.20	2.03	<19	58	<7.8	<0.50	ALOREVA 08	Negative	Negative	Negative
ALOREVA 08	3′	<3.9	<1.20	<1.56	<19	63.2	<7.8	<0.50				
ALOREVA 10	0	<3.9	<1.20	1.60	<19	129	<7.8	<0.50	ALOREVA 10	Negative	Negative	NS
ALOREVA 10	3	<3.9	<1.20	<1.56	<19	145	<7.8	<0.50				
ALOREVA 11	0	<3.9	<1.20	2.91	<19	130	<7.8	0.67	ALOREVA 11	Negative	NS	Negative
ALOREVA 11	3′	<3.9	<1.20	<1.56	<19	222	<7.8	<0.50				

[a]Patient A section of the table shows the study of cytokines at the inclusion visit (V0) and the final visit (3 or 3′).

[b]Patient B section of the table shows the results from blood samples of patients included in the clinical trial. Assay was performed by Cellerix S.A. (Facility no. 4190-E, Madrid, Spain, http://www.pharmatech.es/empresas/cellerix-sl).

Abbreviations: A, blood obtained before treatment with e-ASCs; ALOREVA, ALOREVA clinical trial (Allogeneic Stem Cells Derived From Lipoaspirates for the Treatment of Rectovaginal Fistulas Associated to Crohn's Disease); B, blood obtained after first treatment with e-ASCs; C, blood obtained after second treatment with e-ASCs; e-ASCs, expanded allogeneic adipose-derived stem cells; IL, interleukin; IFN, interferon; NS, no sample obtained; RV, reference value of laboratory; TNF, tumor necrosis factor.

of a severe Crohn's disease flare-up that required biological therapy. The remaining seven patients received the second dose of stem cells (40 million). After this second dose, two patients healed, and two did not. The other three patients, with worsening of the disease, did not complete the trial because of the introduction of biological therapy. In 9 out of 10 patients, the fistula was cured at some point during the trial, although the fistula reopened or the patient had to be excluded from the study in seven cases. The final efficacy rate was 60%, because three out of five patients were cured, with the fistula remaining closed at 52 weeks follow-up.

Discussion

Rectovaginal fistula treatment in Crohn's disease remains frustrating. Treatment depends not only on patient characteristics and the severity of symptoms, but also on the number of fistula tracks, the relationship of the fistula to the sphincter muscles, and the associated perianal disease [22–24]. The healing rate after treatment may range between 0% and 100%, with recurrence rates between 0% and 75% [25]; also, complete closure of the fistula is never achieved in a substantial number of patients.

This trial was conceived and designed following the encouraging results of three previous trials—phases I, II, and III—for the treatment of complex anal fistula [15, 16, 18, 26]. A healing rate of 71% in the phase II trial and of up to 83.33% in the phase III study (when the therapy was applied by selected medical teams) with almost no risk of incontinence and a low recurrence rate were achieved [16, 18]. These three prior studies were conducted to evaluate the treatment of perianal fistula and rectovaginal and enterocutaneous fistula (both included only in the phase I trial) with autologous adipose-derived stem cells. In contrast, in the present trial we used adipose-derived stem cells obtained from a healthy donor (allogeneic adipose-derived stem cells) to treat CRRVF.

Allogeneic mesenchymal stem cells obtained from healthy donors are accessible to more patients and eliminate the need to collect fat tissue from patients. Thus, such easily available treatment can be rapidly administered by using the resources of a cell bank, is safe, and could come at a lower cost and avoid minor surgery for patients.

Because the primary endpoint was to analyze the safety and feasibility of e-ASCs to treat CRRVF, patients were closely monitored to detect any AEs related to the stem-cell therapy. We found no sign of AEs and, as with other authors before [27, 28], concluded that e-ASCs are safe and also feasible.

In order to learn about issues of rejection to e-ASCs, patient cytokine levels were monitored throughout the study (seven times overall). No significant differences were detected, leading us to conclude that no allergic reactions were developed, as has been communicated previously by other authors [29].

Although this clinical trial did not have the statistical power to confirm efficacy objective, and despite the lack of a control arm, the efficacy outcomes were nevertheless interesting. The tertiary aim of the study was to obtain preliminary data on efficacy necessary for a subsequent phase II trial design. Nevertheless, the studies had a high rate of exclusions during the follow-up (50%) and for tacrolimus or cyclosporine administration during the follow-up, and per protocol results appear to differ from the intention-to-treat results, so our results must be taken with caution. Moderate healing rates were obtained.

According to the Spanish regulatory authorities (AEMPS), in this specific pilot study it was considered imperative to discontinue biological medications before beginning the study. The double aim pursued with this limitation was to avoid masking any potential allergic reactions and, on the other hand, to prevent the benefits of the medication from being interpreted as the result of the stem-cell therapy. Unfortunately, we observed a high rate of Crohn's disease flare-ups that could have been avoided had this medication not been discontinued. As a consequence, 5 out of 10 patients were not able to complete the trial because biological (anti-TNF) drugs were required to control a severe flare-up at some point in the study. After this observation, we believe that the potential interaction between biological treatment and stem cells should be studied in depth to clarify whether both can be used together.

When anti-TNF medications have been used to treat fistulous Crohn's disease, a protocol based on an induction first dose followed by maintenance doses has been advocated [30–33]. Similarly, we hypothesized that stem-cell-therapy results could be optimized by adopting a protocol with periodic application of cells; we believe we could improve results and keep the Crohn's-related fistula closed with periodic application of e-ASCs to prevent rectovaginal fistulas from reopening.

The novel therapy described here could provide significant benefits in comparison with current clinical management; the proposed treatment is a simple outpatient procedure, and therefore, postoperative hospitalization is reduced. Also, the treatment is minimally invasive, ensuring greater patient comfort.

Conclusion

To our knowledge, this is the first published study in which allogeneic stem cells to treat rectovaginal fistula in Crohn´s disease seem to be a feasible and safe treatment. We believe additional studies are necessary to confirm the efficacy profile of the e-ASC strategy in a controlled design and to establish the best doses. This may be the beginning of a future line of clinical trials, pending the results from a phase III clinical trial to assess the efficacy and safety of allogeneic e-ASC for the treatment of perianal fistulizing Crohn's disease (ADMIRE Study).

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Author Contributions

M.G.-A.: conception and design, administrative support, data analysis and interpretation, manuscript writing, final approval of manuscript; M.D.H.: financial support, provision of study patients, data analysis and interpretation, final approval of manuscript; C.G.-G.: provision of study patients, collection and/or assembly of data, final approval of manuscript; P.d.l.Q.: administrative support, collection and/or assembly of data, and final approval of manuscript; H.G., T.G.-H., and J.T.: provision of study patients, final approval of manuscript; D.G.-O.: conception and design, financial support, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

D.G.-O. is a member of the Advisory Board of Tigenix S.A.U.; M.G.-A. and D.G.-O. have applied for two patents related to Cx401 and Cx601 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). D.G.-O. is a compensated consultant. The other authors indicated no potential conflicts of interest.

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