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***Observational Study***

**Recurrent anal fistulae: Limited surgery supported by stem cells**

Garcia-Olmo D *et al*. Stem-cell therapy – recurrent anal fistulae

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**Author contributions:** Garcia-Olmo D and Guadalajara H as main authors (equally) designed the study, performed the surgical procedures and acquired and analysed all data, they supervised the project, and wrote the first draft of the paper; Rubio-Perez I collaborated in the analysis and interpretation of data, critically revised the main text and content (including grammar and style), and wrote the final version of the paper; Herreros-Marcos MD collaborated in design and conception of the study and performed the surgical interventions; Quintana P contributed to the first acquisition of data from patients in the outpatient clinics and their analysis; Garcia-Arranz M provided cell resources and managed all regulatory and legal aspects related to the study, participating in design and conception, he collaborated in the revision of contents related to cell behaviour and physiology; all authors revised and approved the final version to be published.

**Ethics approval:** This work has been conducted recruiting patients under a “Compassionate Use” Program. In Spain this kind of treatment is legislated by the Royal Spanish Decree 1015/2009, 19th of July. We had to make an individual request for every patient to the Spanish Agency for Medicines and Health Products (AEMPS). Consents were signed by the attending surgeon and the patient.

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**Conflict-of-interest:** García-Olmo D and Garcia-Arranz M, have applied for two patents relatedwith this study entitled “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). García-Olmo D is a member of the Advisory Board of Tigenix SAU. This manuscript has not been published nor has been presented as a podium/poster presentation in a scientific meeting.

**Data sharing:** Participants gave informed consent for data sharing patient. Nevertheless the risk of identification is very low. The research has also the commitment of keeping the anonymity of the patients.

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**Abstract**

**AIM:** To study the results of stem cell therapy under a “Compassionate Use” Program for patients with recurrent anal fistulae.

**METHODS:** Under controlled circumstances, and approved by European and Spanish laws, a “Compassionate Use” program allowed the use of stem cell therapy for patients with very complex anal fistulae. Candidates had previously undergone multiple surgical interventions that had failed to resolve the fistula, and presented symptomatic recurrence. The intervention consisted of limited surgery (with closure of the internal opening), followed by local implant of stem cells in the fistula-tract wall. Autologous expanded adipose-derived stem cells were the main cell type selected for implant. First evaluation was performed on the 8th postoperative week; outcome was classified as Response or Partial Response. Evaluation a year after the intervention confirmed if complete healing of the fistula had been achieved.

**RESULTS:** Ten patients with highly-recurrent and complex fistulae were treated, 80% male, with a mean age of 49 years (range: 28-76). Seven were non-Crohn’s fistulae, and three were Crohn’s-associated fistulae. Previous surgical attempts ranged from 3 to 12. Two patients presented with preoperative incontinence (Wexner scores of 12 and 13 points respectively). After the intervention, six patients showed clinical response on the 8th postoperative week, with a complete cessation of suppuration from the fistula. Three patients presented a partial response, with an evident decrease in suppuration. A year later, six remained healed (60%), with complete re-epithelization of the external opening. Postoperative Wexner Score scored zero in six cases. The two patients with previous incontinence improved their scores from 12 to 8 points and 13 to 5 points respectively. No adverse reactions or complications related to stem cell therapy were reported during the study period.

**CONCLUSION:** Stem cells have already proven to be safe and useful for the treatment of anal fistulae. Even in the most complex and recurrent cases healing can be achieved, sparing fecal incontinence risk, and even improving previous scoring.

**Keywords:** Fistula-in-ano; Crohn’s disease; Cell therapy; Adipose derived stem cells; Compassionate-use

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**Core tip:** Our group has been working with adipose stem cells (ASC) for several years now, performing various clinical trials. Patients with very complex fistulae, multiple previous surgeries and treatment failure are generally not able to enter these studies despite they would benefit the most, even as their “last chance” of cure. We present the results of a “Compassionate Use” Program, which enabled the application of stem cell therapy to these patients, under strict regulations. Ten patients were treated, and after one year of follow-up, we concluded that ASC are effective and safe, and 60% of the patients achieved complete healing.

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**INTRODUCTION**

A limited surgical treatment in recurrent perianal fistulae often results in new recurrence, whereas there is a high risk of fecal incontinence if an extensive surgical treatment is performed[1-3]. The use of stem cells as medicines to treat complex fistulae is a promising area of research[4,5], for they may help to regenerate damaged perianal tissue. Especially in Crohn’s Disease, the presence of these cells could favour healing through anti-inflammatory and immunomodulatory effects[6-9]. Various randomized controlled trials using stem cells for the treatment of anal fistulae have already been conducted, and all of them show an excellent safety profile. Nevertheless, the real efficacy is yet hard to assess[5]. A recent Spanish study revealed that the mean annual global cost of conventional treatments for patients with Crohn’s disease and perianal fistulae is over 8000€/year[10].

According to current regulatory issues at the time (2002), our Team started a clinical trial process in order to test the ability of adipose-derived stem cells (ASC) to improve healing in complex perianal fistulae, including those associated to Crohn’s disease. The chosen cell source was adipose tissue because the harvesting process for ASC following liposuction was simple and could be performed in our on-site laboratory[11]. To the date, we’ve finished a complete clinical trial process: a pilot study[12] and a Phase II[13] and Phase III clinical trials[14]. Summarizing results, although a complex perianal fistula is the worst scenario, we observed satisfactory healing in our patients, without associated fecal incontinence and therefore excellent prospects. Nowadays, we are developing novel clinical trials directed to test different strategies in order to improve our results[5].

However, during this period of time, some patients with a multi-recurrent anal fistula did not meet the strict eligibility criteria of clinical trials or were scheduled in control groups. The only option to treat these fistulae with stem cells was by “Compassionate Use”. To achieve this, the European regulatory laws and the Spanish Medicine Agency guidelines were followed in order to obtain regulatory permissions. It is important to remark that, under a Compassionate-Use Program, the surgical technique and the cells’ lineage could be tailored for each patient, reinforcing the possibilities of cure, as opposed to the clinical trial setting. In these special cases, we performed minimal surgical maneuvers (limited surgery) directed to the conditioning of the surgical field, followed by implant of cells, in order to improve healing. This strategy enabled us to avoid the anal sphincter injury and also facilitated cell homing[6].

The aim of this paper is to report our experience in a clinical trial- complementary “Compassionate-Use” Program, and discuss about the possible clinical uses of stem cells in the future, focusing on the treatment of complex and recurrent perianal fistulae.

**MATERIALS AND METHODS**

We present an observational study, including 10 patients (8 males and 2 females) with recurrent perianal fistulae who had previously undergone at least 3 surgical interventions (maximum: 12, average: 6.2 times), with failure to resolve the fistula. Mean age was 49 years, and ranged from 28 to 76 years (Table 1). Seven patients presented complex non-Crohn’s fistulae (four were Parks type III)[15] and three patients had Crohn’s-associated perianal fistulae. Two of these patients complained of fecal incontinence at the moment of enrollment in this study, with a Wexner Score[16] > 10.

Autologous expanded adipose-derived stem cells (eASC) were selected in eight cases. Another case was treated using stromal vascular fraction (SVF) and in the last one, allogeneic adipose derived stem cells (Allo-eASC) were employed.

Both eASC (autologous and allogeneic) and SVF protocols were approved by the institutional (La Paz University Hospital) Ethics Committee in accordance with Spanish law, and by the Spanish Medical Agency according to European Medicine Agency (EMA) guidelines. All patients signed a detailed informed consent prior to any intervention, which included permission for data publication. Our institutional Committee on Human Experimentation (La Paz University Hospital) supervised all interventions performed. All ethical standards were in accord with those of the Helsinki Declaration (1975).

***SVF from lipoaspirate***

The liposuction was performed by a plastic surgeon and obtained80 to 100 ml of fat. Phosphate buffered saline (GIBCO BRL, Paisley, United Kingdom) was used to wash the raw lipoaspirate and remove local anesthetics and cells. To extract the cellular fraction, the washed fat was digested with type I collagenase (GIBCO BRL) at a final concentration of 0.075% in saline solution at 37 ºC for 45 min.

Collagenase was inactivated with Dulbecco’s modified Eagle’s medium (GIBCO), this solution included fetal bovine serum (10% v/v). Cellsin suspension were then centrifuged for 10 min (250 g) and phosphate buffered saline was used again to wash the pellet. Centrifugation was repeated and afterwards the remaining erythrocytes were lysed by treating the suspension with ammonium chloride 160 mmol/L for 10 minat room temperature. To conclude the cellular extraction, a final wash and a filtration of the product through a 40 µm nylon mesh was performed.

Before injection, cells were suspended in sterile Ringer-lactate solution (Griffols S.A., Barcelona, España). Morphologic determinations and phenotypic analyses were performed during product obtention. Data are partly published in Garcia-Olmo *et al*[12]. The cell viability registered was always over 95%. Trypan-blue (Sigma, St Louis MO, United States) was used for this determination.

***Autologous stem cell expansion and preparation for implantation***

The released cellular fraction (SVF) was seeded at 2x104-3x104 cells/cm2. Culture was carried out in DMEM medium with 10% of Fetal Bovine Serum (GIBCO) and 1% Ampicillin/Streptomycin. No additional supplements were added. The atmospheric conditions were 37ºC under a 5% CO2 atmosphere.

Cells were re-plated once an 80% confluence was confirmed; their prior detachment was performed by trypsinization (Trypsin: EDTA; GIBCO).This cycle was repeated up to 3 times until the required number of cells for implantation was obtained. Due to logistics and personal issues, in two cases the cells were then frozen for preservation.

Morphologic determinations and phenotypic analyses were performed during expansion. Flow cytometry was the technique employed. Mycoplasma was discarded by Myco Alert Mycoplasma Detection Kit (Cambrex-United States). Data are partly published in Garcia-Olmo *et al*[12].

At least one week before the surgical intervention was scheduled, expanded ASC were prepared (washed with PBS, trypsinized and centrifuged).Their viability was checked (> 95%), and finally the cells were resuspended in Ringer-lactate solution (Griffols S.A., Barcelona, España)at the desired volume and concentration (depending on the fistula) for their immediate use.

***Allogeneic stem cell cell expansion and preparation for implantation***

These cells were manufactured from donors by Tigenix SAU (Madrid, Spain) according to EMA permissions and regulations from healthy donors. The expansion protocol, in general lines, was similar to that of autologous procedures.

***Treatment procedure and evaluation of healing***

All surgical procedures were performed at La Paz University Hospital (Madrid), by the same team of surgeons, belonging to the Colorectal Surgery Unit.

In all cases, a deep curettage of the tracts was first performed, and then the ASC suspension (50%) was injected through a long fine-needle into the tract walls. The injections were superficial, that is, not deeper than 2 mm. In seven cases, the fistulous tract was sealed with fibrin glue (Baxter Inc., Spain) containing part (1 ml) of the cells. The fibrin glue was used as a sealant to finalize the procedure in order to ensure cells remained in the fistulous area. The main reason for injecting a percentage of the ASC into the fibrin glue was to have a reservoir in the area so they could act for longer. However, recent investigations are showing that cells alone suffice to obtain a therapeutical effect[17,18].

 In very complex perianal fistulae, a partial fistulectomy was performed without removing intra-sphincteric tracts. The closure of the internal opening was achieved by stitches in six cases and by a mucosal advancement-flap in three cases. In the remaining patient a fistulotomy was performed.

***Treatment outcomes***

A first evaluation was performed on the 8th postoperative week, and a final evaluation was scheduled a year after the procedure (although patients attended the outpatient clinic in between, at variable intervals). Response was defined as a complete cessation of suppuration on week 8, despite a complete re-epithelization was not achieved. Partial response was defined as an evident decrease in suppuration. Healing was defined as no suppuration from the external orifice, achieving even a complete re-epithelization after 1 year of follow-up. These intervals of time for follow-up were selected following published data about the best periods for long-term fistula follow-upevaluation[19].

**RESULTS**

Of the 10 highly-recurrent perianal fistulae treated, 6 showed a clinical response 8 wk after the procedure and 3 a partial response. A year later, 6 remained healed (60%), with the external opening being completely epithelialized (Table 1). Postoperative results of Wexner Score for Incontinence[16] were zero in 6 cases. In the two patients with previous fecal incontinence the scoring improved from 12 to 8 and 13 to 5, respectively. No adverse reactions or complications related to stem cell therapy were reported during the study period.

No statistical relationships have been established between the use of Fibrin Glue, surgical approach or cell lineage, due to the small number and variability of patients.

**DISCUSSION**

Following strict regulations, we treated 10 patients with recurrent perianal fistulae, achieving a 90% response and a complete healing after a year in 6 cases (60%), with no associated incontinence. Moreover, in two cases previous incontinence was reduced. It is important to remark that the performance of this therapeutic strategy does not produce injury to the anal sphincter, because intrasphincteric-tract resection is not required. Despite this is not a randomized controlled trial, we can observe similar results to those already published[12-14].

ASC enlarge the therapeutic arsenal for anal fistulae, and can be considered an interesting tool for the regeneration/repair of wounds or chronically damaged tissues. The specific mechanism of action of ASC is still under study, but it has been widely demonstrated that these cells improve healing[6]. Two different biological effects are responsible of this healing effect: proliferation and differenciation on the one hand, and immune regulation and local suppression of inflammation on the other[6].

According to the EMA, ASC treatments in the EU should be administered only under clinical trials or other controlled conditions such as ”Compassionate-use” Programs. It is important to remark that all clinical uses of stem cells out of these regulatory conditions are considered illegal. This is clearly stated in the law RD 1015/2009. In this way, in February 2011, the EMA published a report on stem cell-based medicinal products. It expressed concerns about the unregulated use of medicinal products containing ASC.

In this context, and due to the former, one of the limitations of our study is the small number of patients included. We believed that a limited surgical treatment supported by ASC could be beneficial for these patients; but only those that didn’t meet the inclusion criteria or were scheduled in control groups of our clinical trials could be selected for the present study.

Various randomized controlled trials (RCTs) using ASC for the treatment of anal fistulae have been conducted, and all of them show an excellent safety profile. Nevertheless, the real efficacy is hard to assess. To the date, we have identified 11 published papers including data on stem cell-based treatment of anal fistulae (Table 2).

The first one was published in 2003[11] and the last one very recently, in 2013[20]. Eight of them have been published by Spanish groups[11-14,17,21-23], two other papers come from South Korea[20,24] and one from Italy[25]. The majority refer to ASC treatment of Crohn’s disease-related fistulae. The Italian study was the only one to select Bone Marrow as ASC source for the treated fistula[25]. The rest of the studies employed Autologous or Allogeneic cells from adipose tissue. In all studies cells were expanded, and a wide range of doses applied (Table 2). Except for the Italian study[25], all procedures included closure of the internal opening, and in all cases the cell injections were intra-lesional. Over three hundred patients have been enrolled in these studies and the most important result is the assurance of the excellent safety profile of stem cells: no serious cell-related adverse events were described. Regarding efficacy, results show very different profiles, but we could say that around 40%-60% of patients achieved healing (Table 3).

On another basis, the cost of the treatment is an important issue. Nowadays, the production cost of expanded ASC (Good Manufacturing Practice compliant) can range from 8000-12000€. The production cost of ASC without expansion could reduce the expenses to 3000-4000€. This data were reported in our Phase II study[13]. We estimate that a high scale industrial production could significantly reduce the expenses.

A reasonable strategy that we propose, considering the high cost of cell expansion and our previous results, would be to apply a first treatment with SVF, freeze a portion of the cells obtained, and propose a second treatment with expanded cells in cases achieving no response after 8 wk.

Treatment of recurrent fistulae is a difficult surgical challenge. In these patients, the pursuit of healing usually involves multiple operations, with a subsequent perianal scarring and distortion. In the most complex cases, the condition is worsened by accompanying fecal incontinence. Therefore, these individuals are progressively more difficult to treat, resulting in the exasperation of both the patient and the surgeon[26]. In these cases wound healing is a critical issue, and indeed, new approaches are needed. For the reasons outlined earlier, we believe that once available, ASC will fulfill a clear and previously unmet medical need, helping to improve the healing and hence the quality of life of patients with recurrent perianal fistulae.

In conclusion, limited surgery supported by ASC may constitute as a new therapeutic strategy in the treatment of recurrent fistulae.

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**comments**

***Background***

The concept of stem cell therapy came from the possibility of obtaining an immature cell that could differentiate into a specific lineage if placed in the correct environment. One of the first examples was the transplantation of stem cells in hematologic patients, which could achieve the regeneration of normal marrow, and is now widely used. Following this idea, researchers raised the question of whether or not there could be different stem cells for other organs, or even embryonic cells that could develop to any of them. The study and therapeutic use of these types of cells could be the answer for many incurable injuries and diseases.

***Research frontiers***

Complex and recurrent anal fistulae (whether associated or not to Crohn’s disease), constitute an important surgical problem, which can be difficult to solve. Many strategies have been proposed to achieve healing, including different surgical techniques, fibrin glues, plugs, *etc.* The application of stem cells in the fistula tract pursuits the “closure” of the fistula by stimulating the regeneration of the tissue, both by direct growth and immunomodulatory effects. The exact mechanisms by which stem cells induce healing are still under investigation.

***Innovations and breakthroughs***

An increasing number of Randomized Controlled Trial shave tested the application of adipose-derived stem cells (ASC) in perianal fistulae, with variable rates of success. In the present study, we applied Adipose Stem Cells to the “worse” patients, those with recurrent fistulae despite multiple previous treatments and interventions. In these desperate cases, even a partial response to the treatment was a success, as patients’ distress was a constant after so many failures. ACS was remarkably effective and we achieved a 60% of healing after a year of follow-up.

***Applications***

This study, and various others in a randomized controlled trial setting, suggest that the surgical application of stem cells in anal fistula tract is a potentially therapeutic strategy that could resolve even the most recurrent and complex fistulae. In the same direction, ACS is being used for the regeneration of skin, cartilage, bone, cornea, endothelium, *etc.* Other organs such as the heart, the lung and the nervous system have raised high expectations in the field. Research continues, and new applications are sure to develop in the near future.

***Terminology***

Anal fistula is an abnormal conduct communicating the anal canal with the perianal skin. The fistula tract usually breaks through the sphincters, and can have multiple ramifications. If one of the openings is blocked, an abscess occurs, which can worsen the condition. Drainage of these abscesses and surgical attempts to close the fistula can damage the muscle of the sphincters and cause fecal incontinence. The former, associated to suppuration and pain are the most common symptoms, creating a permanent discomfort for patients. Adult (somatic) stem cells are undifferentiated cells that can be found in differentiated tissue (such as bone, fat, muscle, *etc.*) and have the potential to give rise to the specialized cell types present in the tissue from which they originate. A stem cell fulfills 3 characteristics: self-renewal capacity, differentiation potential and *in vivo* engraftment capacity. Stem cell therapy: use of stem cells to replace those from damaged or diseased tissue. The source of the cells can either be the patient (autologous), another individual (allogeneic) or an animal (xenogeneic).

***Peer review***

A good and interesting study even though it includes only ten patients. However, the results are very useful to speculate about the best treatment of recurrent complex fistulae now days. It could be interesting if a randomized cross-over multicenter study will be able to conclude in the future on the really significant good results with stem cell therapy in the complex anal fistulae.

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**Table 1 Study data**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID** | **Gender** | **Age** | **Crohn's disease** | **Park's classification** | **Previous surgical attempts** | **Initial incontinence score (Wexner)** | **Surgical technique** | **Fibrin glue** | **Cells** | **Response 8th week** | **Incontinence score 8th week** | **Healing one year after** |
| 1 | Male | 58 | No | III | 8 | 1 | Flap + deep curettage | No | eASC | Yes | 0 | Yes |
| 2 | Male | 43 | No | II | 4 | Unknown | IO closure + partial fistulectomy | Yes | eASC | Yes | 4 | No |
| 3 | Male | 76 | No | III | 5 | 0 | IO closure + deep curettage | Yes | eASC | Partial | 0 | No |
| 4 | Male | 57 | No | III | 12 | 0 | IO closure + deep curettage | Yes | eASC | Yes | 0 | Yes |
| 5 | Female | 45 | Yes | IV (multiple tracts) | 6 | 12 | IO closure + deep curettage | Yes | eASC | Partial | 8 | Yes |
| 6 | Male | 35 | Yes | II (stenosis) | 5 | Unknown | IO closure + deep curettage | No | eASC | No | 0 | No |
| 7 | Male | 40 | Yes | III | 3 | Unknown | IO closure + deep curettage | Yes | eASC | Yes | 0 | Yes |
| 8 | Male | 59 | No | II | 11 | 13 | Flap + deep curettage | No | eASC Allog | Partial | 5 | No |
| 9 | Male | 50 | No | I | 3 | Unknown | Fistulotomy | Yes | eASC | Yes | 0 | Yes |
| 10 | Female | 28 | No | III | 5 + ileostomy | Not evaluable  | Flap + partial fistulectomy | Yes | SVF | Yes | Not evaluable | Yes |

patients’ characteristics and interventions performed. IO: Internal opening; eASC: expanded adult stem cells; Allog: Allogeneic; SVF: Stromal vascular fraction.

**Table 2 Published clinical experience on stem cell therapy for anal fistulae**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Ref.** | **Year**  | **Condition** | **Study design** | **Cell source** | **Cell quantity (dose)** | **Intervention model** |
| García-Olmo *et al*[11] | 2003 | Recto-vaginal fistula in Crohn’s disease | Case report | Autologous eASC | 1 x 107 | Single arm |
| García-Olmo *et al*[12] | 2005 | Enterocutaneous, recto-vaginal, perianal fistula in Crohn’s disease | Phase I | Autologous eASC | 1 x 107-3 x 107 re-suspended in fibrin glue | Single arm |
| García-Olmo *et al*[13] | 2009 | Perianal fistula with or without Crohn’s disease | Phase II | Autologous eASC | Not specified | Two arms: fibrin glue, fibrin glue + eASC |
| García-Olmo *et al*[22] | 2010 | Recto-vaginal fistula in Crohn’s disease | Case report | Allogenic eASC | Not specified | Single arm |
| Ciccocioppo *et al*[25] | 2011 | Enterocutaneous and complex perianal fistula in Crohn's disease | Case report | Expanded Autologous bone marrow | 5 x 107 | Single arm |
| Cho *et al*[24] | 2012 | Perianal fistula in Crohn's disease | Phase I | Autologous eASC | Not specified | Single arm: dose escalation study |
| Herreros *et al*[14] | 2012 | Complex perianal fistula without Crohn’s disease | Phase III | Autologous eASC | 2 ×107 then 4 x 107 if no effect | Three arms: fibrin glue, eASC, fibrin glue + eASC |
| Herreros *et al*[14] | 2012 | Complex perianal fistula without Crohn’s disease | Observational | Autologous eASC | 2 ×107 then 4 x 107 if no effect | Three arms: fibrin glue, eASC, fibrin glue + eASC |
| Guadalajara *et al*[23] | 2012 | Perianal fistula with or without Crohn’s disease | Observational | Autologous eASC | Not specified | Two arms: fibrin glue, fibrin glue + eASC |
| Portilla *et al*[17] | 2012 | Perianal fistula in Crohn's disease | Phase I/II | Allogeneic eASC | 2 ×107 then 4 x 107 if no effect | Single arm |
| Yong Lee *et al*[20] | 2013 | Perianal fistula in Crohn’s disease | Phase II | Autologous eASC | Depending on the fistula. Re-dosing (1.5 times) if no effect | Single arm |

Description of studies and interventions. SAE: Serious adverse events (those requiring hospital admission > 24 h); eASC: expanded adult stem cells.

**Table 3 Published clinical experience on stem cell treatment for anal fistula**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Ref.** | **Procedure** | **Number of patients treated** | **Healed** | **Follow-up (mo)** | **Recurrence** | **SAE** |
| García-Olmo *et al*[11] | Closure of IO. Injection in site, without fibrin glue. | 1 | 1 | 3 | 0 | 0 |
| García-Olmo *et al*[12] | Cells re-suspended in fibrin glue. Injection in site. | 9 | 6 | 12 | Not specified | 0 |
| García-Olmo *et al*[13] | Closure of IO. Injection in site | Fibrin glue: 25. Fibrin glue + eASC: 24 | Fibrin glue: 3. Fibrin glue + eASC: 17 | 12 | Fibrin glue: 0. Fibrin glue + eASC: 2 | 4 (only one related to fibrin glue, others unrelated) |
| García-Olmo *et al*[22] | Closure of IO. Injection in site, without fibrin glue. | 1 | 1 | 36 | 1 | 0 |
| Ciccocioppo *et al*[25] | Four injections in site. | 10 | 7 | 12 | 0 | 0 |
| Cho *et al*[24] | Closure of IO and fibrin glue. Injection in site | 9 | 3 | 15 | 0 | 0 |
| Herreros *et al*[14] | Closure of IO. Injection in site | eASC: 64. Fibrin glue + eASC: 60. Fibrin glue: 59 | eASC: 27. Fibrin glue + eASC: 24. Fibrin glue: 23 | 6 | eASC: 0. Fibrin glue + eASC: 4. Fibrin glue: 0 | 4 unrelated to study treatment |
| Herreros *et al* .[14] | Closure of IO. Injection in site | Not specified | eASC: 57%. Fibrin glue+ eASC: 52.4%. Fibrin glue: 37.3% | 12 | Not specified | 1 unrelated to study treatment |
| Guadalajara *et al*[23] | Closure of IO. Injection in site | Fibrin glue: 13. Fibrin glue + eASC: 21 | Fibrin glue: 3. Fibrin glue + eASC: 10 | 38 | Fibrin glue: 1. Fibrin glue + eASC: 5 | 0 |
| Portilla *et al*[17] | Closure of IO. Injection in site, without fibrin glue. | 24 | 9 | 4 | Not specified | 2 unrelated to study treatment |
| Yong Lee *et al*[20] | Injection in site and fibrin glue. | 43 | 27 | 12 | 4 | 0 |

Procedures and outcomes. IO: Internal opening; SAE: Serious adverse events (those requiring hospital admission > 24 h); eASC: expanded adult stem cells.