



Review Article

Adipose tissue-derived stem cells: a new approach to the treatment of Crohn's disease-associated perianal fistulae

Mónica Maria Ramos Tavares*, Laura Elisabete Ribeiro Barbosa

Porto University Medical School, Porto, Portugal

ARTICLE INFO

Article history:

Received 7 February 2018

Accepted 30 March 2018

Keywords:

Crohn's disease

Fistula

Adipose tissue

Stem cell

ABSTRACT

Crohn's disease has an ever-increasing prevalence and incidence, with about 20% of patients developing perianal fistula with significant impact on their quality of life.

Despite the medical and surgical treatments currently used, Crohn's-related fistula treatment continues to pose a challenge due to the low rates of efficacy associated with high recurrence rates.

Recent clinical trials have shown promising results regarding safety and efficacy of local treatment of this condition with the use of adipose tissue-derived mesenchymal stem cells. Besides being pluripotent and poorly immunogenic, they have immunomodulatory and anti-inflammatory properties, which combined, may accelerate healing.

Our main objective is to summarize the clinical trials we found, highlighting the efficacy rates of this therapy and the main limitations we found in the analysis of the results.

We conclude that, in perianal fistulas refractory to conventional therapies, the treatment with adipose tissue-derived mesenchymal cells is safe with promising results that may change the current paradigm of Crohn's related fistula treatment.

© 2018 Sociedade Brasileira de Coloproctologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Células estaminais derivadas do tecido adiposo: uma nova abordagem no tratamento de fístulas perianais associadas à doença de Crohn

RESUMO

A incidência e a prevalência da doença de Crohn têm aumentado e, ao longo do decurso da doença, cerca de 20% dos doentes irão desenvolver fístulas perianais com impacto significativo na sua qualidade de vida.

Apesar dos tratamentos médicos e cirúrgicos utilizados atualmente, o tratamento destas fístulas continua a constituir um desafio com baixas taxas de eficácia e com elevadas taxas de recorrência.

Palavras-chave:

Doença de Crohn

Fístula

Tecido adiposo

Células estaminais

* Corresponding author.

E-mail: monicamariaramos@gmail.com (M.M. Tavares).

<https://doi.org/10.1016/j.jcol.2018.03.004>

2237-9363/© 2018 Sociedade Brasileira de Coloproctologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Ensaios clínicos recentes têm demonstrado resultados promissores em termos de segurança e eficácia de tratamentos locais destas fístulas com o recurso a células estaminais mesenquimatosas derivadas do tecido adiposo que, além de pluripotentes e pouco imunogênicas, têm capacidades imunomoduladoras e anti-inflamatórias capazes de promover o processo de cicatrização.

O objetivo desta revisão sistemática é sumarizar os ensaios clínicos encontrados, realçando as taxas de eficácia desta terapêutica e as principais limitações na análise dos resultados.

Concluimos que, nas fístulas perianais refratárias às terapias convencionais, o tratamento com células estaminais mesenquimatosas derivadas do tecido adiposo é seguro e com resultados promissores que podem mudar o paradigma atual do tratamento das fístulas complexas associadas à Doença de Crohn.

© 2018 Sociedade Brasileira de Coloproctologia. Publicado por Elsevier Editora Ltda. Este é um artigo Open Access sob uma licença CC BY-NC-ND (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Crohn's disease (CD) is a chronic and progressive inflammatory disease that may involve the entire gastrointestinal tract, the incidence and prevalence of which have increased, both in the adult and pediatric population, especially in Western countries.^{1,2} It is estimated that in Europe one million people have CD.³

Despite these epidemiological data, its etiology is still unknown, and does not have curative treatment in the light of current knowledge, with many patients being even refractory to conventional treatments.^{1,2}

Genetic, environmental and microbiological factors, together with morphological and functional alterations of the intestinal barrier associated with a deficient immune response, seem to be involved in the development of the disease.¹

Regarding the symptoms of active CD, these include intestinal and extra-intestinal manifestations that hinder the differential diagnosis with other pathologies in many situations.⁴

In addition, through the course of the disease, more than 20% of patients will develop perianal fistulae. These occur in approximately 12% of patients with ileal disease, in 15% of patients with ileocolic disease, in 41% of patients with non-rectal colic disease, and in 92% of patients with colon and rectum disease.⁵

These CD-associated fistulae have a pathophysiology that is very similar to cryptoglandular fistulae; however, they have a particularity related to its etiology. Most of them do not result from an infectious focus, but from an abnormal activation of the immune system that triggers chronic inflammation and consequent transmural lesion of tissues that can culminate in a fistula.⁶

Their categorization can be made using both Parks et al.'s, and the American Gastroenterology Association classifications.⁴ Most of them are complex anorectal fistulae with a high rate of associated complications, including pain, infection, and abscess formation, often difficult to control therapeutically, requiring antibiotics and corticosteroids.^{7,8} All this has a great impact on patients' quality of life, not

only because of the unpredictability of the effectiveness of conventional treatments, but also because of the high rates of recurrence. On the other hand, these patients are still at an increased risk of developing colorectal carcinoma.⁸

The medical treatment available includes antibiotics and immunosuppressive/immunomodulatory drugs such as thiopurines and tacrolimus and, more recently, anti-tumor necrosis factor alpha (anti-TNF α) drugs, such as infliximab and adalimumab.⁴

In case of medical treatment failure, surgical treatment through fistulotomies or fistulectomies is indicated, often associated with the placement of seton, and requirement of surgery in several stages. Fibrin glue (FG), injected directly into the fistula path, insertion of a plug into the fistula, application of negative pressure patches and/or endorectal flaps can also be used. In addition, there are some drugs available for topical application, such tacrolimus and even intralesional injections of infliximab.⁵

However, the treatment with these drugs, either isolated or in combination, has variable success rates, between 30% and 80%. Many times, the complete closure of the fistula or the prevention of abscesses, or of relapses are not achieved.⁴

Currently, anti-TNF α biological drugs are the first-line treatment for CD-associated complex fistulae. However, this therapy seems to only cause a decrease in the inflammatory response, rather than support active tolerance and promote long-term tissue regeneration; thus, the results in terms of sustained healing are also unsatisfactory.⁹

On the other hand, due to its mechanism of action, this type of therapy increases the risk of development of severe infections and congestive heart failure, and promotes the reactivation of viruses such as that of hepatitis B. Its immunogenicity has also contributed to a decrease in its efficacy when used in the long term. For this reason, it is commonly administered with other therapies, including corticosteroids and immunomodulators.¹⁰

Over the past three decades, we have seen great progress in regenerative medicine and stem cell biology.¹¹ The use of these cells through the transplantation of hematopoietic cells for the systemic treatment of CD has been described since 1993. However, more recent studies have resorted to other types of stem cells, the so-called mesenchymal cells, both to

systemically treat active forms of disease, and for the local treatment of complex fistulae associated with it.¹²

In 2009, the US Federal Drug Administration (FDA) approved the use of Prochymal (Osiris Therapeutics, Inc., USA) for the treatment of graft versus host disease, and for the treatment of CD.¹³ However, it is in the field of local treatment with the use of these cells that there has been more development in recent years.¹⁴

The mesenchymal stem cells were first described in 1974 by Friedenstein, but it took about two decades for this term to be widely adopted.¹⁵ They are pluripotent cells, able to differentiate into different cell types, and are present in all body tissues.⁴

However, all these cell populations appear to be intrinsically different from each other, according to the differences found in the substances they secrete.¹⁵ The main harvesting sources are the spinal cord, and adipose tissue.⁴ These two subpopulations have similar immunomodulation functions in cell differentiation, but adipose tissue-derived mesenchymal cells (ADSCs) show a more potent modulation capacity by increasing cytokine secretion, and their harvesting method is also more accessible, and allows a greater amount of cells to be harvested, with less discomfort for the patient.^{1,12}

These cells' immunomodulatory function is based on their capacity to down-regulate mucosal immune reactivity and promote tissue healing. Some studies have shown that they also have the ability to inhibit *in vitro* T cell proliferation, including T-helper 1 and 17, and to inhibit lymphocyte proliferation by the activation of a cell apoptosis pathway.² These cells seem to be able to revert the imbalance present in the extracellular matrix metabolism of the basal membrane epithelial cells of the intestine, caused by the increase of the metalloproteinases in relation to its inhibitors, described in the inflammatory bowel disease.¹⁶ They also have anti-inflammatory properties, and the ability to resist for long periods in *ex vivo* conditions.^{4,9} In addition to their ability to regenerate tissues, they promote angiogenesis and prevent fibrosis.¹⁷

Although the mechanisms are still poorly understood, studies appear to demonstrate that these cells affect tissues, which are proximity targets, and are not dependent on direct cell-to-cell contact. In addition, they have the ability to stay in the areas of injury/inflammation even when systemically given. That is, its efficacy does not rely on graft adhesion, but on its ability to create a favorable environment through the secretion of bioactive molecules.^{6,12}

Due to all these characteristics, ADSCs have been used in a growing number of diseases for more than ten years, especially in cases of tissue degeneration, post-radiotherapy lesions, hematopoietic and post-transplant diseases, as well as in refractory chronic inflammatory diseases, and in fibrotic and fistulizing diseases.¹⁷

An increasing number of publications have demonstrated the effectiveness of ADSC therapy in a variety of preclinical models, including acute lung injury, septic shock, and acute myocardial infarction. A number of small clinical trials have also investigated the efficacy and safety of treatment with these cells, including chronic heart failure, acute myocardial infarction, hematological diseases, renal diseases,

joint diseases, diabetes, liver diseases, and graft versus host disease.^{15,18}

Mesenchymal stem cells are poorly immunogenic, and have 90% of similarity of phenotypes; thus, prior to treatment, the patient does not need to undergo previous induction/conditioning with chemotherapy.² They do not have Histocompatibility Major Complex (HCM) class II molecules nor costimulatory molecules capable of activating T cells. They also have low levels of CMH5 class I molecules.

This low immunogenicity, coupled with easy technique for cell collection and expansion, can allow its mass production with the use of healthy donors. However, some *in vitro* studies have shown that these cells are not intrinsically immunoprivileged. Under certain conditions, allogeneic cells are able to stimulate T cells, which may result in transplant rejection.²

Other concerns related to the use of these cells are their neoplastic potential derived from their proliferative capacity, the greater susceptibility of individuals to infection due to their immunomodulatory effects, cell embolism, the emergence of culture-reagent-related zoonoses, and acute immunogenicity or chronic inflammation of the cells themselves.¹⁸

Regarding the neoplastic potential, recent studies show that there is no risk of malignant transformation of these cells, either through *in vitro* chromosome aberrations, or *in vivo* tumor induction, even in long-lasting cultures.¹

In 2003, the first case treated with this technique in the local treatment of perianal fistulae was published. It was a 33-year old woman with CD that was diagnosed eleven years before, with perianal suppuration and rectovaginal fistula that did not improve with Infliximab and seton placement. After a local injection of 9×10^6 ADSCs, the surgical wound was closed within a week with no signs of incontinence. The patient remained asymptomatic for three months.⁶

Since then, several clinical trials have been developed to evaluate the safety and efficacy of local treatment with ADSCs in CD-associated complex fistulas. Although the results are promising, this is a very recent therapeutic strategy, hence the importance of this review to systematize the clinical trials performed, its main results, and limitations.

Methods

This systematic review included randomized and non-randomized human trials, electronically searched on PubMed, EMBASE and Open Access Journals, with the terms "Crohn's disease", "Fistula", "Adipose tissue" and "Stem cells" until October 31, 2017. The research was carried out independently by the authors and, in total, nine articles were found.

A search was also conducted in the ClinicalTrials.gov database of ongoing clinical trials on this subject. In this database, 11 clinical trials were found, two of which are still in progress, and three were canceled due to protocol failures or difficulties in recruitment (NCT01378390, NCT01314092, NCT01623453).

In addition, the relevant bibliographic references of the selected articles were analyzed and articles of systematic review and meta-analysis on the use of stem cells in the

treatment of inflammatory bowel disease and of fistulae associated or not with CD were investigated.

Our main objective is to summarize the clinical trials found, highlighting the efficacy rates and the main limitations found in the analysis of the results.

Results

The first clinical trial published on the treatment of CD-associated complex fistulae using ADSCs dates back to 2005. It was a Phase I trial carried out in Spain involving four participants. Amounts between 3 and 30×10^6 of autologous ADSCs were injected according to the cells culture growth rate in nine fistulas. After 8 weeks, a total closure rate of the external hole of 75%, and a significant reduction of drainage in the remaining inoculated fistulae were obtained. No serious adverse events were recorded, and the follow-up period was 22 months.¹⁹

The same authors published again in 2009. This time, the publication was the results of a controlled, randomized, multicenter, phase II clinical trial with 49 participants (14 had complex CD-associated perianal fistulae, and 35 had complex perianal fistulae of cryptoglandular origin).²⁰

Patients were randomly divided into two groups, A and B, with 25 and 24 participants, respectively. Group A received intralesional treatment with FG, and group B with FG, and 20×10^6 of autologous ADSCs. In non-closed fistulae after eight weeks, based on the group, a second dose of FG, or FG with 40×10^6 ADSCs was administered. At the end of eight weeks after the last injection, fistula closure was recorded in 71% of patients in group B, and in only 16% of patients in group A. The proportion of patients who had fistula closure was similar between the patients with and without CD. At the end of one year, the recurrence rate in group B was 17.6%. No serious adverse events associated with administration of ADSCs have been reported.²⁰

In 2013, the results of an open, multicenter, phase I/IIa pilot trial conducted in six Spanish hospitals were published. A sample of twenty-two patients received 20×10^6 allogeneic ADSCs in the path of a CD-associated draining fistula. Subsequently, 40×10^6 ADSCs were injected into fistulae whose closure was not complete within 12 weeks. At the end of this period, the external hole closure rate was 36.8%, and at the end of 24 weeks it was 53.3%, with 66.6% of patients reporting a reduction in the number of draining fistulas. The follow-up period was between 6 and 8 months, and two serious adverse events associated with the treatment were recorded: pyrexia and perianal abscess.¹⁰

Also in 2013, the results of an open-label, multicenter phase I clinical trial conducted in South Korea between 2008 and 2009 with a sample of 10 patients with more than one CD-associated fistula were published. Participants were sequentially distributed into three groups who received an injection with an amount of autologous ADSCs proportional to the size of the fistula. The first group of three patients were injected with 10×10^6 cells/mL, which was shown to be a safe amount at the end of four weeks of follow-up. In the second group of three patients, 20×10^6 cells/mL were administered. After four weeks, the administration of this amount was also

shown to be safe, and in the third group of three patients, 40×10^6 cells/mL were injected. After the injection, the path of the fistulas was filled with a solution of thrombin and fibrinogen. At the end of 8 weeks, the rate of complete closure of the fistula was 33% (two patients in group 2 and one patient in group 3). The remaining five patients had only partial closure. Patients with complete closure showed no signs of recurrence at the end of 8 months of treatment.²¹

In another open-label phase II study, also carried out in South Korea, and published in 2013, 43 participants with CD-associated complex fistulae were given autologous ADSCs intralesionally into a FG matrix, in an amount proportional to the size of the fistulae. Patients who did not achieve fistula closure after 8 weeks received a second injection of ADSCs containing 1.5 times more cells compared to the first injection. At 8 weeks after the last injection, fistula closure was achieved in 82% of the patients ($n=27$), with a recurrence rate of 11.5% at the end of one year of follow-up.²²

More recently, in 2016, the results of a phase I/II clinical trial were published in which 10 patients with CD-associated rectovaginal fistulae were treated. The protocol also included the intralesional injection of 20×10^6 ADSCs in the submucosa of the vaginal walls and in the path of the fistula. In cases of non-re-epithelialization of the vaginal and/or rectal walls with no drainage, a second injection with 40×10^6 ADSCs was given. Only half the participants completed the study, with a healing rate of 60% in a one-year follow-up period. No serious adverse reactions or signs of rejection were observed.²³ In the same year, the results of an open, multicenter, dose-escalation pilot study that included a sample of 6 patients divided into two groups, who received allogeneic ADSCs intralesionally, based on the size of the fistula. To group A ($n=3$), 10×10^6 ADSCs were injected and, to group B ($n=3$), 30×10^6 ADSCs. In case of complete closure of the fistula after 8 weeks, the patients were followed up for an additional period of 6 months. The healing rate was 50% (2 patients in group A, and one patient in group B). In all patients, the closure was sustained during the follow-up period. There were no serious adverse reactions associated with the treatment.²⁴

Also in 2016, they published the results of a randomized, double-blind phase III clinical trial that enrolled 212 patients, selected between 2012 and 2015, from 49 hospitals with complex and draining CD-associated perianal fistulae. In this trial, patients were randomly divided into 2 groups, and then stratified according to the concomitant treatment (anti-TNF α , immunomodulators, or both). Group A, with 107 patients, was treated with an injection of 120×10^6 ADSCs, and the remaining 105 were the placebo group, who were injected with only 24 mL of saline solution. At the end of 24 weeks, 51% of patients in group A vs. 36% in the placebo group achieved remission combined with clinical and magnetic resonance imaging criteria. The follow-up period was of 6 months, during which 5% of serious adverse reactions occurred in group A: anal abscesses.²⁵

In July 2017, the results of a phase I clinical trial with a sample of 12 patients with complex CD-associated perianal fistulae were published. The fistulae were treated with autologous ADSCs, involved in a bioabsorbable matrix, applied through a plug, not by intralesional injections. At the end of 6

months, 10 of the 12 patients (83%) had radiologically proven fistula closure.²⁶

In addition to the previously described clinical trials, the results of which have already been published, two trials are still in progress. A randomized, double-blind, controlled-phase, Phase III clinical trial entitled “Adult Allogeneic Expanded Adipose-derived Stem Cells (eASC) for the Treatment of Complex Perianal Fistula(s) in Patients With Crohn’s Disease (ADMIRE-CD-II)”, registered under code NCT03279081 in the ClinicalTrials.gov database. This trial has not reached the recruitment phase, but is estimated to be completed in 2021. Its objective is to evaluate safety and efficacy of intraleisional injection of allogeneic ADSCs in the closure of complex CD-associated fistulae, evaluated at week twenty-four for a follow-up period of 52 weeks. Patients receiving concomitant medical treatment may be enrolled in this study, provided they are in stable doses.

There is a clinical trial, still in the recruitment phase, that has been in progress in Italy since 2015, and is scheduled to be completed by December 2018. It is an open-label, non-randomized, phase II study with registration NCT02403232 in the ClinicalTrials.gov database, with the title “Autologous Adipose-derived Stem Cells (ASCs) for the Treatment of Perianal Fistula in Crohn’s Disease: A Pilot Study (ASPEFIC1)”, the aim of which is to evaluate the efficacy of treatment of CD-associated perianal fistulae with autologous ADSCs. The main objective is to achieve a healing rate of 65% at the end of one year, compared with 25% of the ACCENT II study - the largest and most important multicenter randomized study involving infliximab published in 2002, to evaluate the impact of maintenance therapy every 8 weeks in patients with CD-associated refractory fistulae.

Discussion

From the analysis of the clinical trials described above, it can be concluded that the local treatment of CD-associated complex fistulas with ADSCs, besides being safe, seems to have positive and promising results with low recurrence rates, contributing to a significant improvement in quality of life, and reduced risk of developing cancer.

However, one of the major limiting factors in most clinical trials is the absence of a control group, and a reduced number of participants, coupled with a high dropout rate.

On the other hand, it should also be noted that in all the trials only adults were included, with perianal fistulas classified as complex, refractory to conventional treatment and, in most cases, with diameter/collections of less than 2 cm.

Another major limitation, especially in the first studies, was the exclusion of patients receiving other types of therapies, concomitantly or even afterwards. This prevented the evaluation of potentiating therapeutic effects in these trials, and even the safety of the various therapies when they are simultaneously used in these trials.

From the technical point of view, we still have the different methodologies used, both in the harvesting and in the technique of ex vivo expansion of the stem cells. This may be a very important factor, because some studies demonstrate that the expansion technique may decrease the

differentiation capacity of ADSCs, and that cryopreservation and thawing techniques may negatively influence their immunomodulation capacity and biodistribution.²⁷

On the other hand, they describe that different subpopulations of these cells may have different functional abilities, and therefore, have a confusing role in the results obtained in the trials. Therefore, it may be important to investigate an in vitro biomarker associated with the function of in vivo ADSCs.

In most cases, the fistulas were subjected to curettage techniques and closure of the internal hole, prior to local treatment with ADSCs; however, the technique used and even the number of cells injected was too heterogeneous, and it is not possible to define a minimum efficacious dose of ADSCs.

It appears that the type of ADSC transplantation does not seem to influence fistula closure rates, but the risk of malignancy should be addressed when healthy donor cell banks are searched. On the other hand, allogeneic transplantation may increase the cost-effectiveness of this technique, reducing time-consuming, and overcoming some obstacles that are inherent to the autologous harvesting process, including the reduction of adipose tissue in advanced stage CD patients.

Another factor that is confounded in the analysis of the results is the use, in some clinical trials, of a fibrin glue matrix that alone has the capacity to activate fibroblasts and endothelial cells, having an important role in collagen synthesis, and angiogenesis, which are fundamental to the healing process.

Regarding the time from the last administration of the treatment to the complete closure of the fistula, it varied between 8 and 24 weeks, and the closure criteria ranged from clinical to imaging or combined criteria.

No serious adverse events have been reported in most clinical trials, so it can be concluded that this is a safe treatment, at least when used in adults. However, although recent studies show that there is no risk of malignization of ADSCs, the maximum follow-up period in most trials analyzed was 12 months, which may not be enough to safely evaluate this parameter.

In short, it can be stated that the local treatment of CD-associated fistulae using ADSCs is a safe, minimally invasive procedure that reduces the length of stay and the risk of fecal incontinence compared to the surgical option.

Its administration is also associated with a lower number of serious adverse events, which appear to present higher healing rates and lower recurrence rates, relative to anti-TNF α biological therapies when administered alone, which predicts a greater long-term efficacy rate. This efficacy seems to be superior when the protocol allows the administration of local treatment with ADSCs concomitantly with other therapies, including immunomodulators and anti-TNF α .²⁵

Conclusion

CD-associated complex fistulae are a current problem that deserves our attention, not only because of the impact this condition has on the quality of life of patients, but also because their treatment remains a challenge.

There has been an increasing number of studies on the safety and efficacy of local treatments using ADSCs. However, although the results seem promising, the various clinical trials present very diverse study designs and methodologies,

making it difficult to clarify the role of this new therapy in the treatment algorithm of CD.

Therefore, additional clinical trials, with a larger number of participants and with less restrictive selection criteria are necessary, allowing the administration of combined therapies. To create a safe protocol for harvesting, culturing and expanding the ADSCs is important, as well as to define more uniform remission/healing criteria, and a minimum effective amount of cells to be given.

In spite of all the limitations mentioned above, by analyzing the results of clinical trials performed to date, it is legitimate to state that we may be facing a new era in the treatment of CD-associated complex fistula.

The local treatment with ADSCs, besides having promising healing rates, has fewer complications and recurrence rates, presenting several advantages when compared with surgical treatment or administration of biological therapies.

The use of ADSCs banks of healthy donors may further reduce the cost-effectiveness of this technique, which, together with the advantages described above in comparison to conventional treatments, may contribute to reduce the economic impact of this condition on the national health system.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

- Flores AI, Gómez-Gómez GJ, Masedo-González A, Martínez-Montiel MP. Stem cell therapy in inflammatory bowel disease: a promising therapeutic strategy? *World J Stem Cells*. 2015;7:341–51.
- Gazouli M, Roubelakis M, Theodoropoulos G. Stem cells as potential targeted therapy for inflammatory bowel disease. *Inflamm Bowel Dis*. 2014;20:952–5.
- Cosnes J, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology*. 2011;140:1785–94.
- Al-Maawali AKS, Nguyen P, Phang PT. Modern treatments and stem cell therapies for perianal Crohn's fistulas. *Can J Gastroenterol Hepatol*. 2016;2016:1651570.
- American Gastroenterological Association Clinical Practice Committee. American Gastroenterological Association medical position statement: perianal Crohn's Disease. *Gastroenterology*. 2003;125:1503–7.
- Cho YB, Yoon KJ, Yoon SN, Song KH, Kim DS, Jung SH, et al. Long-term results of adipose-derived stem cell therapy for the treatment of Crohn's fistula. *Stem Cells Transl Med*. 2015;5:32–7.
- Zhang X, Zhang Y, Wang W, Wei Y, Deng H. Mesenchymal stem cell to treat Crohn's disease with fistula. *Hum Gene Ther*. 2018;28:534–40.
- Cao Y, Ding Z, Han C, Shi H, Cui L, Lin R. Efficacy of mesenchymal stromal cells for fistula treatment of Crohn's disease: a systematic review and meta-analysis. *Dig Dis Sci*. 2017;62:851–60.
- Ciccocioppo R, Corazza GR. Mesenchymal stem cells for fistulising Crohn's disease; 2016. Available at: <https://thelancet.com> [accessed 02.09.17].
- de la Portilla F, Alba F, García-Olmo D, Herreras JM, González FX, Galindo A. Expanded allogeneic adipose-derived stem cells (eASCs) for the treatment of complex perianal fistula in Crohn's disease: results from a multicentre phase I/IIa clinical trial. *Int J Colorectal*. 2012;28:313–23.
- Dave M, Mehta K, Luther J, Baruah A, Dietz AB, Faubion WA Jr. Mesenchymal stem cell therapy for inflammatory bowel disease: a systematic review and meta-analysis. *Inflamm Bowel Dis*. 2015;21:2696–707.
- Duran NE, Hommes DW. Stem cell-based therapies in inflammatory bowel disease: promises and pitfalls. *Ther Adv Gastroenterol*. 2016;9:533–47.
- Syed BA, Evans JB. Stem cell therapy market. *Nat Rev Drug Discov*. 2013;12:185–6.
- Martínez-Montiel MP, Gómez-Gómez GJ, Flores AI. Therapy with stem cells in inflammatory bowel disease. *World J Gastroenterol*. 2014;20:1211–27.
- Hawkey CJ, Hommes DW. Is stem cell therapy ready for prime time in treatment of inflammatory bowel diseases. *Gastroenterology*. 2017;152:389–97.
- Buzhor E, Leshansky L, Blumenthal J, Barash H, Warshawsky D, Mazor Y, et al. Cell-based therapy approaches; the hope of incurable diseases. *Regen Med*. 2014;9:649–72.
- Voswinkel J, Francois S, Simon JM, Benderitter M, Gorin NC, Mohty M, et al. Use of mesenchymal stem cells (MSC) in chronic inflammatory fistulizing and fibrotic diseases: a comprehensive review. *Clin Rev Allergy Immunol*. 2013;45:180–92.
- Lalu MM, McIntyre L, Pugliese C, Fergusson D, Winston BW, Marshall JC, et al. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS ONE*. 2012;7:e47559.
- García-Olmo D, García-Arranz M, Herreros D, Pascual I, Peiro C, Rodríguez-Montes JA. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Dis Colon Rectum*. 2005;48:1416–23.
- García-Olmo D, Herreros D, Pascual I, Pascual JA, Del-Valle E, Zorrilla J, 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.
- Cho YB, Lee WY, Park KJ, Kim M, Yoo HW, Yu CS. Autologous adipose tissue-derived stem cells for the treatment of Crohn's fistula: a phase I clinical study. *Cell Transplant*. 2013;22:279–85.
- Lee WY, Park KJ, Cho YB, Yoon SN, Song KH, Kim DS, et al. Autologous adipose tissue-derived stem cells treatment demonstrated favorable and sustainable therapeutic effect for Crohn's fistula. *Stem Cells*. 2013;31:2575–81.
- García-Arranz M, Herreros MD, González-Gómez C, de la Quintana P, Guadalajara H, Georgiev-Hristov T. Treatment of Crohn's-related retrovaginal fistula with allogeneic expanded-adipose derived stem cells: a phase I-IIa clinical trial. *Stem Cells Transl Med*. 2016;5:1–6.
- Park KJ. Allogeneic adipose-derived stem cells for the treatment of perianal fistula in Crohn's disease: a pilot clinical trial. *Colorectal Dis*. 2016;18:468–76.
- Panés J, García-Olmo D, Van Assche G, Colombel JF, Reinisch W, Baumgart DC, et al. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial; 2016. Available at: <https://thelancet.com> [accessed 02.09.17].
- Dietz AB, Dozois EJ, Fletcher JG, Butler GW, Radel D, Lightner AL, et al. Autologous mesenchymal stem cells, applied in a bioabsorbable matrix, for treatment of perianal fistulas in patients with Crohn's disease. *Gastroenterology*. 2017;153:59–62.
- Algeri M, Conforti A, Pitisci A, Starc N, Tomao L, Bernardo ME, et al. Mesenchymal stromal cells and chronic inflammatory bowel disease. *Immunol Lett*. 2015;128:191–200.