



Mesenchymal stem-cell therapy for perianal fistulas in Crohn's disease: a systematic review and meta-analysis

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Abstract

Background Mesenchymal stem-cell (MSC) therapy for perianal fistulas in Crohn's disease (CD) remains controversial. We performed this meta-analysis to evaluate the efficacy and safety of MSC therapy for the treatment of perianal fistulas in CD.

Methods Electronic databases were searched for studies that reported efficacy and/or safety of MSC therapy for perianal CD (pCD). We used the metaprop command of the meta package in R and RevMan to assess the efficacy and safety. Subgroup analyses were performed for exploration of heterogeneity regarding all outcomes.

Results After screening, 13 trials were included in our study. RevMan for meta-analysis showed that: (1) patients had healed perianal fistulas after MSC treatment, with an odds ratio (OR) of 2.05 ($P=0.0002$; 95% confidence interval (CI) 1.41, 3.00) vs controls; (2) pelvic magnetic resonance imaging (MRI) used to evaluate the healing of fistulas showed that MSC therapy had a higher healing rate (HR) compared to control (OR = 1.95; $P=0.0007$; 95% CI 1.33, 2.87). R software for meta-analysis showed that 63% (95% CI 0.53, 0.74) of patients achieved clinical healing as a result of local therapy with MSCs. Random-effects pooled rates of clinical response were 30% (95% CI 0.18, 0.48). Pelvic MRI used to evaluate fistula healing showed a HR of 56% (95% CI 0.46, 0.69). The HR with autologous MSCs was higher than with allogeneic MSCs (79% vs 52%; $P<0.05$). Uniform injection of MSCs according to the size of fistulas improve the HR (80% vs 55%; $P<0.05$) compared with fixed-dose MSCs. There was no significant increase in adverse events (OR = 1.14; $P=0.54$; 95% CI 0.75, 1.74) in patients treated with MSCs and no major MSC-related adverse event has been reported so far.

Conclusions Local administration of MSCs is an effective and safe method for pCD. It also represents hope for effective treatment of refractory pCD.

Keywords Crohn's disease · Perianal fistula · Mesenchymal stem cells · Meta-analysis · Efficacy · Adverse effects

Introduction

Crohn's disease (CD) is an immune-mediated chronic recurrent, systemic disease characterized by gastrointestinal inflammation. Perianal fistula is a common complication of CD, and is estimated to affect up to 26–28% of patients in the first two decades after diagnosis [1, 2]. The treatment

of pCD includes conventional medical treatment strategies (e.g., antibiotics and immunomodulators) and anti-tumour necrosis factor agents (anti-TNF), or surgical treatments. However, up to 60–70% of patients relapse after stopping medical treatment, and only a few patients achieve long-term remission [3]. Surgical treatment is associated with problems of poor healing and a high rate of recurrence. In addition, impaired sphincter muscle function following surgery causes postoperative anal incontinence in about 10–35% patients. In some severe cases, fecal diversion may be used to manage CD [4], but recurrence is high with restoration of continuity, and up to 20–40% of these patients will eventually undergo a proctectomy [5]. Therefore, management of refractory pCD and the realization of long-term healing have remained a challenging problem.

In recent years, tremendous progress has been made in the field of regenerative medicine and stem-cell biology.

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Stem-cell therapy has become a hot research topic. Stem cells are a unique group of undifferentiated cells that have capacity of self-renewal and they can be broadly categorized as embryonic or adult-derived stem cells. Among adult stem cells, the best defined cells are the hematopoietic stem cells (HSCs), MSCs, and intestinal stem cells. Given that MSCs are able to down-regulate immune responses, anti-inflammatory properties, and promote tissue healing [6–10], they are most commonly used to treat pCD. MSCs can achieve the reconstruction of intestinal immunity, to avoid immune-mediated intestinal inflammation, and may achieve long-term healing of pCD, significantly improving the quality of life of patients. Therefore, MSCs may serve as an alternative treatment for refractory pCD. Multiple studies have been conducted to assess efficacy and safety of MSC therapy in pCD, but the outcomes still remain controversial. We performed a systematic review and meta-analysis of the literature to determine the efficacy and safety of MSC therapy.

Materials and methods

We followed the preferred reporting items for systematic reviews and meta-analysis (PRISMA) guidelines [11].

Literature search

To identify relevant studies, a comprehensive electronic search of Pubmed, Cochrane Library, Embase, and CNKI was performed from their inception to October, 2018. Combinations of the following search terms were used: (all fields): (“inflammatory bowel disease” OR “Crohn’s disease” OR “Crohn disease”) and (“mesenchymal stem/stromal cells” OR “stem cell” OR “stromal cell”) and (“perianal fistula” OR “Crohn’s perianal fistula”) and any appropriate abbreviations.

Study selection

Inclusion criteria were: (1) human studies; (2) randomized controlled trials (RCTs), non-randomized experimental studies, and so on; (3) established diagnosis of CD perianal fistula by accepted criteria, with no limits on the age and gender of patients; (4) MSCs were used for treatment of pCD; (5) interventions: local therapy with MSCs for pCD; (6) efficacy and/or safety were reported; and (7) the primary endpoint was clinical efficacy (fistula healing, clinical response) of MSCs for the treatment of pCD, and the secondary endpoint was safety. Exclusion criteria were: (1) non-human studies; (2) use of total-body irradiation or myeloablative regimens; (3) not belonging to pCD (e.g., rectovaginal fistula or enterocutaneous fistulas); (4) and efficacy of MSCs as systemic infusion for CD.

Data extraction and quality assessment

One reviewer (Z.H) extracted data through a standardized data collection form, and then, another reviewer (Z.L) checked the data for accuracy. Any inconsistent results were handled by discussion. These forms included: (1) author; (2) journal; (3) study characteristics (year, location, and type of study); (4) patient demographics (number of patients, age); (5) number of healthy controls (if any); (6) type and source of MSCs; (7) efficacy outcome; (8) dosage and modalities of administration; (9) the use of concomitant therapy with anti-TNF; (10) follow-up time and recurrence; (11) refractory disease (defined as patients who had been unresponsive to or unsuitable for all the previous medical treatment including anti-TNF or unsuccessfully treated by surgery; and (12) adverse events (AEs) (defined as an untoward medical occurrence associated with the use of MSCs whether related to the MSCs or not).

Assessment of the quality of studies was performed using the Cochrane risk of bias tool [12].

Statistical analysis

The meta-analysis was performed using the `metaprop` command of the meta package in R (version 3.4.2) [13] and RevMan5.3 (Cochrane Collaboration, Oxford, UK) to assess the efficacy (clinical healing and response) and safety (AEs, studies with a comparison arm were included in the meta-analysis). OR and related 95% CIs were calculated to compare MSCs with control cohorts. Log transformation was used to calculate pooled proportions under the fixed and random-effects model [14]. The Chi-square test ($P < 0.05$ indicating statistical significance) and the I^2 statistic (an I^2 of $\geq 50\%$, $< 50\%$ indicating substantial and low heterogeneity, respectively) were used to qualitatively and quantitatively evaluate statistical heterogeneity, respectively. Subgroup data were analyzed using the Chi-square test at the subgroup analysis, and $P < 0.05$ was considered statistically significant.

Results

Literature search

The initial literature retrieval identified 2644 citations. After reading through the title and abstract to exclude the articles, these not meet the inclusion criteria. Of the final 13 studies selected for inclusion in the review [15–27]. Five studies were RCTs [16, 18, 23, 24, 27], and eight studies were non-randomized experimental studies [15, 17, 19–22, 25, 26].

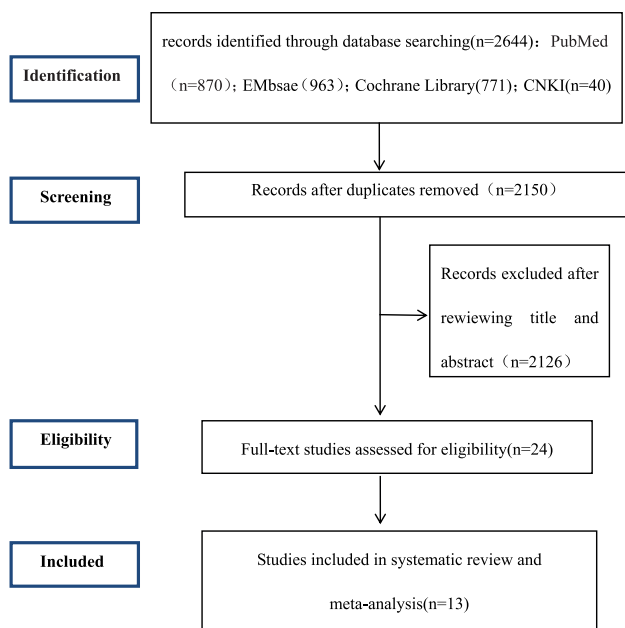


Fig. 1 Flow diagram of included and excluded studies in this meta-analysis

The detailed literature screening process is shown in Fig. 1. Eleven studies used adipose-derived MSCs [15, 16, 18–22, 24–27], and eight studies used autologous MSCs [15–18, 20–22, 26]. Five studies used allogeneic MSCs [19, 23–25, 27] and two studies used bone-marrow-derived MSCs [17, 23]. In seven studies, an-TNF had been stopped since enrolment [15–17, 19–21, 25], and six studies maintained stable dose of anti-TNF during the entire study period [18, 22–24, 26, 27]. In four studies, the dosage of MSCs was determined based on the fistula size [20–22, 25], which was estimated from the diameter and length of the fistula. Eight studies used the fixed dose of MSCs [15–19, 23, 24, 27]. One study used MSC-coated matrix fistula plugs [26]. Study demographics and clinical characteristics are summarized in Table 1.

Comparison of MSCs vs control (local therapy)

Five RCTs [16, 18, 23, 24, 27] reported raw data on fistulas healing of patients with MSCs. Using RevMan for meta-analysis, in a fixed-effects model, MSCs group were more effective for fistulas healing than placebo with an OR of 2.05 (95% CI 1.41, 3.00), with no heterogeneity between the studies ($I^2 = 0\%$) (Fig. 2). Pelvic MRI is a non-invasive, highly accurate examination for the diagnosis and classification of fistula and, therefore, considered to be the imaging gold standard for CD fistulas [28]. It also facilitates early detection of fistula recurrence. Four RCTs using MRI to evaluate the healing of fistulas were pooled [18, 23, 24, 27], yielding an OR of 1.95 (95% CI 1.33, 2.87) for achieving fistulas

healing in patients who received MSCs vs control (Fig. 3), no heterogeneity ($I^2 = 0\%$).

Efficacy of MSCs for pCD (local therapy)

Thirteen studies reported raw data on clinical healing of patients after MSCs therapy with substantial heterogeneity ($I^2 = 67\%$), and the pooled HR was 63% (95% CI 0.53, 0.74) (Fig. 4). Eleven studies [15–17, 19–25, 27] reported raw data on clinical response of patients treated with MSCs. A random-effects pooled rate of clinical response was 30% (95% CI 0.18, 0.48) (Fig. 5). When MRI was used to evaluate the healing of fistulas, the random-effects pooled rate of healing was 56% (95% CI 0.46, 0.69) (8 studies [17–19, 23–27]) (Fig. 6).

Subgroup analysis of efficacy of MSCs for pCD (local therapy)

In a fixed-effects model, autologous MSCs [15–18, 20–22, 26] were more effective for the healing of perianal fistulas than allogeneic MSCs [19, 23–25, 27] (79% vs 52%), with a significantly statistical difference ($P < 0.05$) (Fig. 7). Two studies [17, 23] reported raw data on the outcome of patients with bone-marrow-derived MSCs. The pooled rate of clinical healing was 57% compared to 64% for patients who underwent adipose-derived MSCs, with no statistically significant differences ($P > 0.05$) (data not shown). Six studies [18, 22–24, 26, 27] reported raw data on the healing of pCD that maintained stable dose of anti-TNF during the entire study period, the pooled rate of clinical healing was 63% compared to 61% (in 7 studies [15–17, 19–21, 25]) of patients who had been stopped an-TNF after enrolment, with no statistically significant differences ($P > 0.05$) (data not shown). In a fixed-effects model, the dosage of MSCs [20–22, 25] determined based on the fistula size were more effective for the healing of perianal fistulas than fixed-dose of MSCs [15–19, 23, 24, 27] (80% vs 55%), with a significantly statistical difference ($P < 0.05$) (Fig. 8).

Safety of MSCs for pCD (local therapy)

The included studies demonstrate a good safety and tolerability profile of MSC treatment for pCD. In our study, safety is assessed through AEs analysis: there were no significant increases in AEs (OR = 1.14; 95% CI 0.75, 1.74; $P = 0.54$) when MSC and non-MSC cohorts were compared (Fig. 9). No MSC-related AE has been reported so far. Common AEs such as anal pain, anal bleeding, fever, abdominal pain, diarrhea, and perianal abscess are mostly transient and rarely result in patients needing hospitalization or in disability. In addition, these AEs are associated with local injection procedures of MSCs (e.g., curettage of a fistula and closure

Table 1 MSC therapy for CD patients with perianal fistulas

Study; location	Type of study	N (total)	Cell type and source	Outcome	Results (healed)	Results (respond)
García-Olmo et al. [15] (2005); Spain	Phase I trial	5	Autologous, adipose tissue	Reepithelialization of external opening	3/4 healed at 8 weeks	1/4 incomplete closure at 8 weeks
García-Olmo et al. [16] (2009); Spain	Phase II, trial; RCT	14	Autologous, adipose tissue	Reepithelialization of external opening	5/7 in MSCs at 8 weeks; 1/7 in fibrin glue alone at 8 weeks	2/7 incomplete closure at 8 weeks
Ciccocioppo et al. [17] (2011); Italy	Phase I trial	10	Autologous, bone marrow	Reepithelialization plus absence of collections measured by MRI	6/9 healed at 8 weeks	3/9 incomplete closure at 8 weeks
Guadalajara et al. [18] (2012); Spain	Phase II, trial RCT	8	Autologous, adipose tissue	Reepithelialization plus absence of collections measured by MRI	2/4 in MSCs at 40 months; 1/2 in fibrin glue alone at 40 months	Unknown
De la Portilla et al. [19] (2013); Spain	Phase I/IIa trial	24	Allogeneic, adipose tissue	Reepithelialization plus absence of collections measured by MRI	5/18 healed at 12 weeks	12/19 incomplete closure at 12 weeks
Cho et al. [20] (2013); Korea	Phase I trial	10	Autologous, adipose tissue	Reepithelialization of external opening	3/9 healed at 8 weeks	5/9 incomplete closure at 8 weeks
Lee et al. [21] (2013); Korea	Phase II trial	43	Autologous, adipose tissue	Reepithelialization of external opening	27/33 healed at 8 weeks	5/33 closure by more than 50% of fistula tract with a decrease in drainage of more than 50%
Cho et al. [22] (2015); Korea	Phase II trial	41	Autologous, adipose tissue	Reepithelialization of external opening	21/26 healed at 24 weeks	5/26 incomplete closure
Molendijk et al. [23] (2015); Netherlands	Phase II trial RCT	21	Allogeneic, bone marrow	Reepithelialization plus absence of collections measured by MRI	7/15 in MSCs at 12 weeks; 2/6 in saline at 12 weeks	5/15 incomplete closure in MSCs at 12 weeks; 1/6 in saline at 12 weeks
Panés et al. [24] (2016); Israel	Phase III trial RCT	212	Allogeneic, adipose tissue	Absence of drainage and <2 cm fluid collection on MRI	53/103 in MSCs vs 36/101 in placebo at 24 weeks	18/103 in MSCs vs 20/101 in placebo at 24 weeks
Park et al. [25] (2016); Korea	Phase I trial	6	Allogeneic, adipose tissue	Reepithelialization plus absence of collections measured by MRI	1/5 healed in 8 weeks	4/5 incomplete closure in 8 weeks
Dietz et al. [26] (2017); USA	Phase I trial	12	Autologous, adipose tissue	Absence of drainage and <2 cm fluid collection on MRI	10/12 healed in 6 months	Unknown
Panés et al. [27] (2018)	Phase III trial RCT	212	Allogeneic, adipose tissue	Absence of drainage and <2 cm fluid collection on MRI	58/103 in MSCs vs 39/101 in placebo in 52 weeks	10/103 in MSCs vs 17/101 in placebo in 52 weeks

Table 1 (continued)

Age (year) mean (SD)	Intervention (mean)	Use of concurrent (anti-TNF)	Recurrence	Refractory (drugs/ surgical)	AEs
35.1 ± 2.4	Fixed cells dose 2 × 10 ⁷ MSCs	No	1 year without recurrence	Yes	
43.33 ± 9.90	Fixed cells dose First: 2 × 10 ⁷ MSCs Second: 4 × 10 ⁷ MSCs	No	7 months without recurrence	Yes	
32	Fixed cells dose First: 2 × 10 ⁷ MSCs Second: 2 × 10 ⁷ MSCs	No	1 year without recurrence	Yes	
41.0 ± 9.7	Fixed cells dose First: 2 × 10 ⁷ MSCs Second: 4 × 10 ⁷ MSCs	Yes	Two completely healed patients were followed up for 4 years without recurrence	Yes	
36 ± 9	Fixed cells dose 2 × 10 ⁷ MSCs	No	Unknown	Unknown	
26.5 ± 6.0	Based on the size of the fistula A: 1 × 10 ⁷ MSCs/ml B: 2 × 10 ⁷ MSCs/ml C: 4 × 10 ⁷ MSCs/ml	No	2 year without recurrence	Yes	
26.1 ± 5.6	Based on the size of the fistula 3 × 10 ⁷ MSCs/ml	No	3/26 healed were followed up for 1 years without recurrence	Unknown	
26.2 ± 5.5	Based on the size of the fistula 3 × 10 ⁷ MSCs/ml	Yes	4/24 healed were followed up for 2 years without recurrence	Unknown	
38	Fixed cells dose A: 1 × 10 ⁷ MSCs B: 3 × 10 ⁷ MSCs C: 9 × 10 ⁷ MSCs	Yes	24 weeks without recurrence	Yes	
39.0 ± 13.1	Fixed cells dose 12 × 10 ⁷ MSCs	Yes	Being follow-up	Yes	68/103 in MSCs vs 66/102 in placebo at 24 weeks
32.17 ± 7.96	Based on the size of the fistula A: 1 × 10 ⁷ MSCs/ml B: 3 × 10 ⁷ MSCs/ml	No	Three healed were followed up for 8 months without recurrence	Yes	
18–65	2 × 10 ⁷ cells per plug	Yes	Unknown	Yes	
39.0 ± 13.1	Fixed cells dose 12 × 10 ⁷ MSCs	Yes	Being follow-up	Yes	79/103 in MSCs vs 74/102 in placebo in 52 weeks

MSC mesenchymal stem cells, CD Crohn's disease

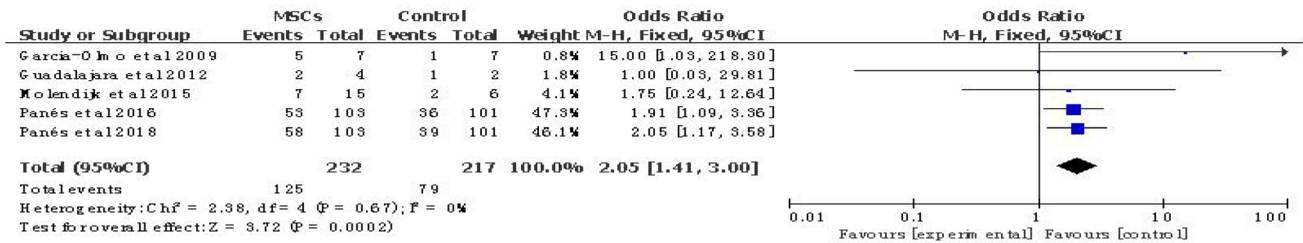


Fig. 2 Efficacy of MSCs and placebo treatment in pCD

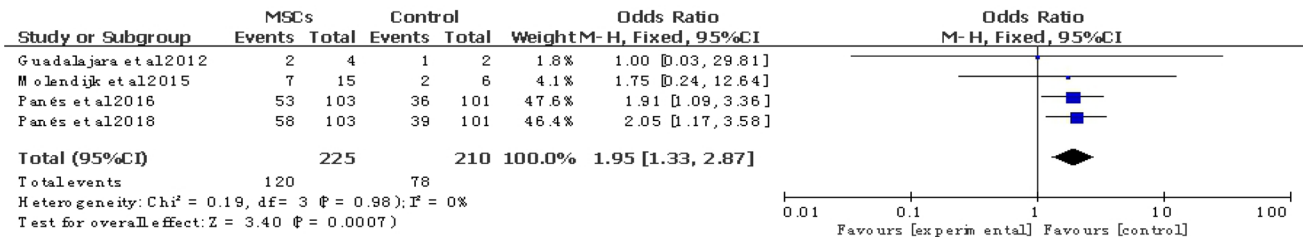


Fig. 3 MRI+reepithelialization of external opening of fistula in evaluation of fistula healing

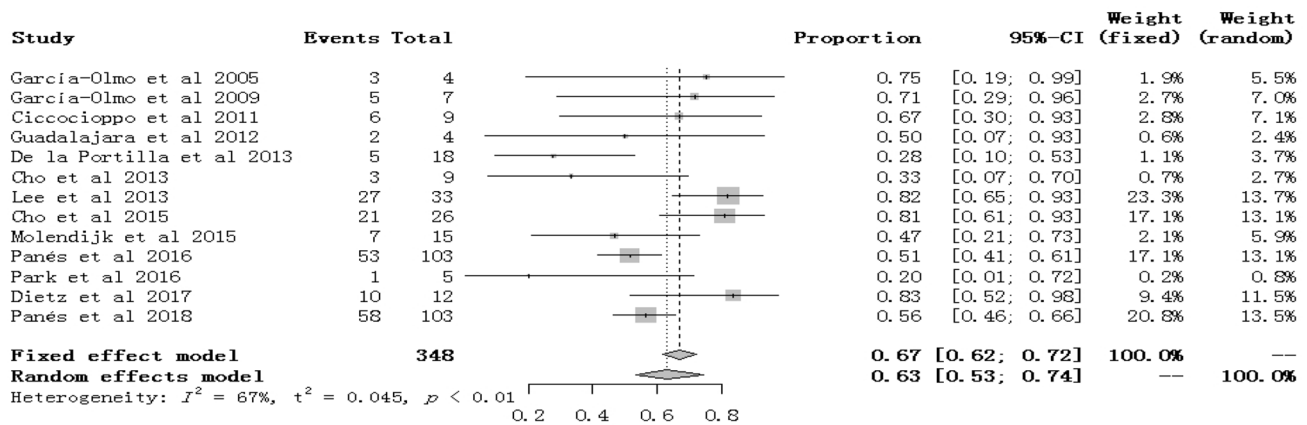


Fig. 4 Forest plot of studies evaluating healing of pCD after local therapy with MSCs

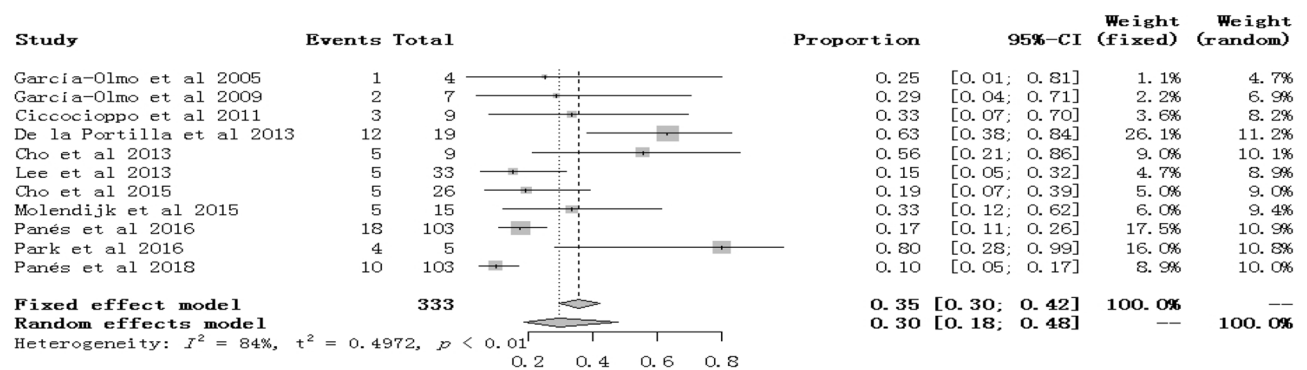


Fig. 5 Forest plot of studies evaluating clinical response of pCD after local therapy with MSCs

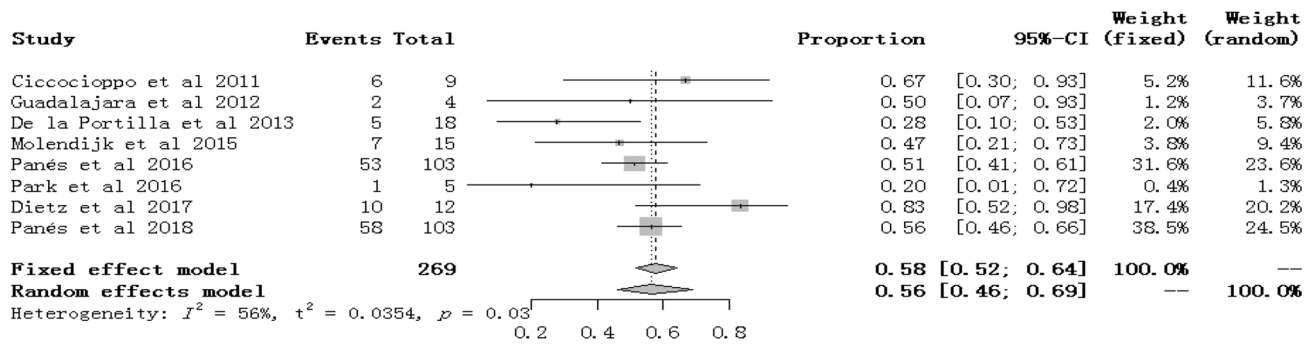


Fig. 6 Forest plot of studies evaluating healing of pCD after local therapy with MSCs (use of MRI)

Fig. 7 Clinical healing (allogeneic or autologous MSCs)

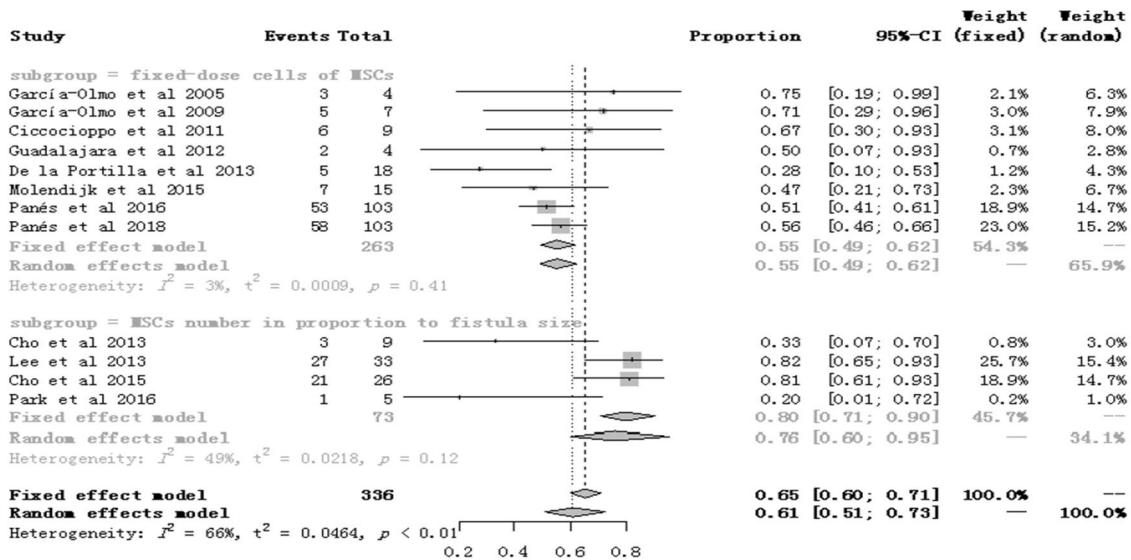
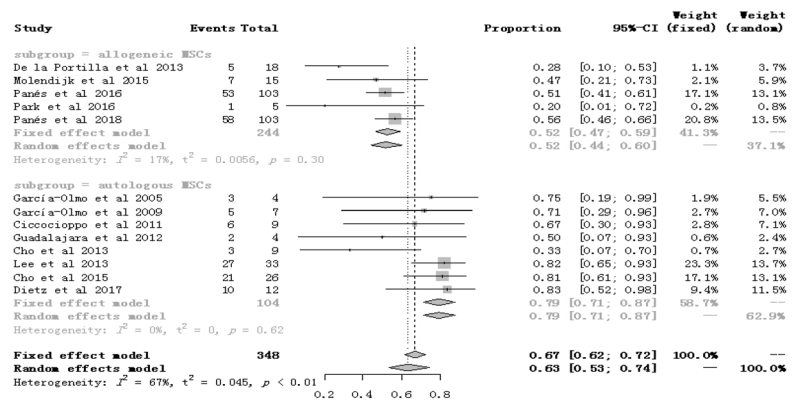


Fig. 8 Clinical healing (different MSC injection modalities)

of an internal opening) or the progression of the disease itself rather than the MSCs themselves. Complications often associated with surgery such as fecal incontinence have not been reported in MSCs' studies. Considering their

immunosuppressive properties, it is not clear whether MSCs increase the risks of malignancy at present. Some animal studies suggested that MSCs could promote tumour growth [29, 30], but this has never been confirmed by clinical

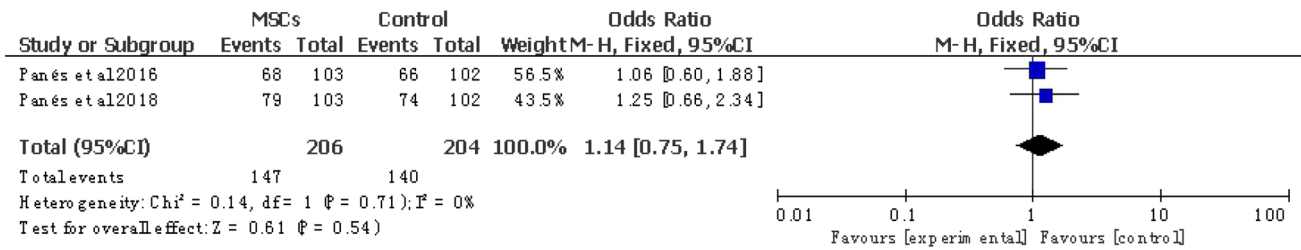


Fig. 9 Pooled OR of adverse events when MSCs were compared with non-MSCs

studies. One patient treated with 1×10^7 MSCs developed an adenocarcinoma of the cecum with peritoneal carcinomatosis > 15 months after the surgical intervention [23]. Baseline and week-12 endoscopy of the rectum revealed no abnormalities. In addition, the last endoscopy of the entire colon and the biopsies taken at that time was completely normal. Moreover, given that MSCs have a very limited lifespan in the body and the patient had a family history of colon cancer, we think that the occurrence of the tumour is associated with genetic factors rather than with MSCs. One patient in the study by de la Portilla et al. [19] was diagnosed with uterine leiomyomata during follow-up. It should be taken into account that uterine fibroids are noncancerous growths of the uterus that often appear during childbearing years and are not associated with an increased risk of uterine cancer. In view of these considerations, this study did not reveal any specific signs of carcinogenicity. A follow-up of up to 4 years suggests that MSCs have no carcinogenic risk [18]. However, we should consider that in this study, the number of patients is small, and there is no standard on the evaluation of safety. Therefore, in the future, we need more patients with long-term follow-up, to thoroughly evaluate the safety of MSC treatment for pCD.

Discussion

In this meta-analysis, we used different softwares to analyze the efficacy of MSCs for pCD. However, there are several limitations: (1) studies used different types and sources of MSCs including adipose tissue, bone marrow from autologous as well as allogeneic; (2) the timepoint of fistula healing fluctuates significantly (8 weeks–40 months); (3) different definitions of fistula healing, only eight studies used pelvic MRI, and the rest defined healing as reepithelialization of external opening; (4) different dosages and modalities of administration; (5) exclusion of younger patients; and (6) through the Cochrane risk of bias tool, we can see that the eight non-randomized experimental studies included are studies with poor stability and quality.

Now, as stem-cell-based treatments are gaining a strong foothold in fistula management worldwide, more clinical

studies are being performed to further ascertain the efficacy and safety of MSC therapy for pCD. Standardization is crucial to assess the efficacy of current and future local treatment strategies. Therefore, with the goal of optimizing this emerging therapy, we need to propose a standardized definition of AEs, and need more objective definitions of the healing of pCD (e.g., endoscopy, C-reactive protein, fecal calprotectin, and imaging including MRI) in future studies.

Several meta-analysis have shown that MSCs are effective and safe for the treatment of pCD [31–35]. However, the results of these studies are still quite different, the explanation for this result may be due to the different sources, different differentiation and regeneration capacity of MSCs and different clinical trials for the isolation of MSCs, different culture programs, and the patient's age, severity of the disease and other factors may also affect the efficacy of MSCs. In our review, autologous, MSCs have shown more promising results than allogeneic MSCs in pCD fistulas. Moreover, the use of autologous MSCs is more acceptable and there is no ethical conflict. However, the possibility of disease-related effects on autologous MSCs must be taken into account. In addition, autologous MSCs are not immediately available upon request, because isolation and expansion of MSCs to a sufficient numbers of cells require weeks that may result in treatment delay. In addition, due to the inflamed intestinal mucosa, patients' nutritional status can be poor and patients are frequently very thin, so it is often difficult to gain access to an adequate amount of MSCs. Allogeneic MSCs, easily obtained from a healthy donor, is a product accessible to more patients. Thus, an easily available treatment can be rapidly administered from a completely validated cell bank and provides an affordable therapy to large numbers of patients. In some trials, MSCs from young healthy donors that may have better immunomodulatory and regenerative potential [36, 37] may be selected to avoid the effects of the underlying autoimmune disease. Therefore, our study shows that the efficacy of autologous MSCs is better than that of allogeneic MSCs, but we need to consider the timeliness and cost-effectiveness of treatment to optimize the treatment. There were no statistically significant differences in the HR in our study when the origin of MSCs used (adipose-derived vs bone-marrow-derived) was considered.

It should be noted that human adipose tissue is plentiful and easily accessible, and contains a large number of stem cells compared to other tissues in the body. Obtaining adipose tissue minimizes side effects on donors (regardless whether patient or healthy donor). To obtain bone-marrow MSCs in some special donors such as patients with a history of myocardial infarction is dangerous. More and more data are becoming available on the differences between bone-marrow- and adipose tissue-derived MSCs, including on their immunomodulatory properties. One study [37] suggests that adipose tissue-derived MSCs might be superior to bone-marrow MSCs in suppressing immune responses in vitro. Therefore, adipose tissue-derived MSCs can be considered a priority in the future. There were only two studies [17, 23] used bone-marrow-derived MSCs, which was a limitation of our study. More RCTs should compare their efficacy and safety. MSCs were used alongside with anti-TNF in some clinical studies, and they share common targets. Their interaction should be studied carefully. In our review, combined anti-TNF with MSCs had no synergistic effect on the healing of fistulas. Most patients included in our study were refractory to anti-TNF. Treatment of anti-TNF does not seem to affect the long-term efficacy of MSCs for refractory patients, and the long-term application of anti-TNF caused by economic problems and AEs. The exposure of MSCs to physiological concentrations of anti-TNF agents does not affect their survival and their inhibitory capacities on peripheral

blood mononuclear cell proliferation [38]. However, whether there is a synergistic effect in combination with MSCs in patients with a response to anti-TNF remains to be determined by further studies.

There are still many unsolved questions concerning the modalities of administration and dosage. In our review, there were two administration methods. One was locally injected MSCs; the fistula tract was thoroughly curetted and irrigated under anesthesia; and the internal opening was closed MSCs which were injected into the submucosa surrounding the internal opening and the fistula track wall. The other method was using MSC-coated matrix fistula plugs. In this study, the volume of injected MSCs proportional to fistula size improved the HR compared with fixed-dose MSCs. CD fistulas usually involve multiple trajectories over a wide range of regions, so treatment of the fistula with a fixed small dose of cells may not be sufficient. We believe that cell number in proportion to fistula size can improve the efficacy, because cells can be injected evenly into the fistula. Therefore, our study provides guidance for the modalities of administration of MSCs. Due to the limitations of studies, it is difficult to provide recommendations on the optimal dose. However, Molendijk et al. [23] reported that local therapy with 3×10^7 MSCs showed superior fistulas healing compared with 9×10^7 MSCs treatment (80% vs 20%. The authors argued that higher cell concentrations could result in a lower survival rate and/or cell function and that a larger number of

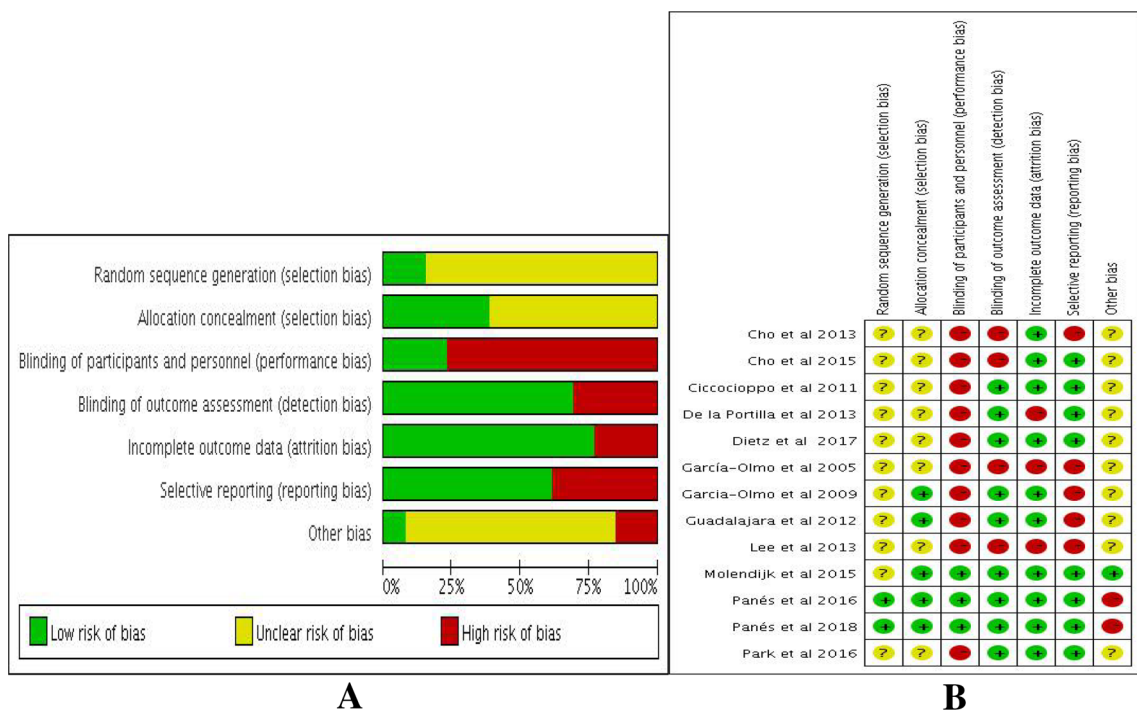


Fig. 10 **a** Overall quality of the included studies assessed by Cochrane risk of bias assessment tool. **b** Risk of bias within studies assessed by Cochrane risk of bias assessment tool

cells could be immunogenic resulting in increased clearance or deactivation of the cells. This provides a guidance in choosing the optimal dosage of MSCs for the treatment of pCD.

Studies included in our meta-analysis used variable definitions of fistulas healing. We showed that when pelvic MRI was used to evaluate fistula healing, the HR fell. We think that healed fistula was defined as complete closure evident on clinical examination and reepithelialization of the external opening of the fistula was not objective and accurate, as it cannot replace the inside of the entire fistula. Pelvic MRI is considered be the imaging gold standard for CD fistula, but MRI is insensitive to reduced fistula caliber. Therefore, we need improved instruments for evaluation of perianal CD fistula healing. Parks' classification of the anatomy of perianal fistulas: (1) intersphincteric; (2) transsphincteric; (3) suprasphincteric; (4) extrasphincteric; and (5) not involving the sphincter complex is a clinically useful classification of perianal fistulas in CD and can help the treating physician to determine optimal management strategy disease. However, due to the limitations of our study, differences in classification of pCD were not analyzed in detail and compared with the differences in response to MSC treatment. RCTs are needed to clarify the mechanism of MSCs for treating CD fistulas by directly comparing the therapeutic effects between MSCs and immunomodulators, biologics, and surgical procedures. Comprehensive consideration of factors that affect the healing of fistulas (such as gender, age, diameter/length of fistula, type of fistula, CD duration, CDAI activity, and other factors), and the selection of appropriate MSCs (type, source, dose, frequency of injection, and mode of delivery) for patients receive the optimal treatment must be further investigated.

Assessment of study quality

There was overall high risk of performance bias, detection bias, and reporting bias in the studies included in the review (Fig. 10a, b).

Conclusions

The results of this study are encouraging, and MSCs appear to offer a safe and effective treatment approach for pCD fistulas. Based on subgroup analysis, autologous, adipose MSCs had relatively higher rates of clinical healing. MSC dosage in proportion to fistula size also had higher rates of clinical healing. The efficacy of MSCs does not seem to be influenced by prior unsuccessful treatment with anti-TNF and may be an attractive option for patients failing this treatment. However, these findings should be interpreted with much caution owing to the limited number of studies

and potential biases. More RCTs are needed to support our conclusions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The present paper as a review of the literature was exempt from approval.

Informed consent For this review of literature, informed consent was not required.

References

- Schwartz DA, Loftus EV Jr, Tremaine WJ et al (2002) The natural history of fistulizing Crohn's disease in Olmsted County, Minnesota. *Gastroenterology* 122:875–880
- Hellers G, Bergstrand O, Ewerth S, Holmström B (1980) Occurrence and outcome after primary treatment of anal fistulae in Crohn's disease. *Gut* 21:525–527
- Domènech E, Hinojosa J, Nos P et al (2005) Clinical evolution of luminal and perianal Crohn's disease after inducing remission with infliximab: how long should patients be treated? *Aliment Pharmacol Ther* 22:1107–1113
- Van Assche G, Dignass A, Reinisch W et al (2010) The second European evidence-based consensus on the diagnosis and management of Crohn's disease: special situations. *J Crohns Colitis* 4:63–101
- Beaugerie L, Seksik P, Nion-Larmurier I et al (2006) Predictors of Crohn's disease. *Gastroenterology* 131:334–335
- Jiang XX, Zhang Y, Liu B et al (2005) Human mesenchymal stem cells inhibit differentiation and function of monocyte derived dendritic cells. *Blood* 105:4120–4126
- Beyth S, Borovsky Z, Mevorach D et al (2005) Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness. *Blood* 105:2214–2219
- Spaggiari GM, Abdelrazik H, Becchetti F, Moretta L (2009) MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: central role of MSC-derived prostaglandin E2. *Blood* 113:6576–6583
- Melief SM, Geutskens SB, Fibbe WE, Roelofs H (2013) Multipotent stromal cells skew monocytes towards an anti-inflammatory interleukin-10-producing phenotype by production of interleukin-6. *Haematologica* 98:888–895
- Melief SM, Schrama E, Brugman MH et al (2013) Multipotent stromal cells induce human regulatory T cells through a novel pathway involving skewing of monocytes toward anti-inflammatory macrophages. *Stem Cells* 31:1980–1991
- Moher D, Liberati A, Tetzlaff J et al (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ* 339:b2535
- Shuster J (2011) Review: Cochrane handbook for systematic reviews for interventions, Version 5.1.0, published 3/2011. Julian P.T. Higgins and Sally Green, Editors. *Res Synth Methods* 2:126–130
- Schwarzer G (2015) Meta: general package for meta-analysis. R Package Version 4.1-0. R Foundation, Vienna

14. Freeman MF, Tukey JW (1950) Transformations related to the angular and the square root. *Ann Math Stat* 21:607–611
15. Garcia-Olmo D, Garcia-Arranz M, Herreros D, Pascual I, Peiro C, Rodriguez-Montes JA (2005) A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Dis Colon Rectum* 48:1416–1423
16. Garcia-Olmo D, Herreros D, Pascual I et al (2009) Expanded adipose-derived stem cells for the treatment of complex perianal fistula: a phase II clinical trial. *Dis Colon Rectum* 52:79–86
17. Ciccocioppo R, Bernardo ME, Sgarella A et al (2011) Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. *Gut* 60:788–798
18. Guadalajara H, Herreros D, De-La-Quintana P, Trebol J, Garcia-Arranz M, Garcia-Olmo D (2012) Long-term follow-up of patients undergoing adipose-derived adult stem cell administration to treat complex perianal fistulas. *Int J Colorectal Dis* 27:595–600
19. de la Portilla F, Alba F, Garcia-Olmo D, Herreras JM, Gonzalez FX, Galindo A (2013) Expanded allogeneic adipose-derived stem cells (eASCs) for the treatment of complex perianal fistula in Crohn's disease: results from a multicenter phase I/IIa clinical trial. *Int J Colorectal Dis* 28:313–323
20. Cho YB, Lee WY, Park KJ, Kim M, Yoo HW, Yu CS (2013) Autologous adipose tissue-derived stem cells for the treatment of Crohn's fistula: a phase I clinical study. *Cell Transpl* 22:279–285
21. Lee WY, Park KJ, Cho YB et al (2013) Autologous adipose tissue-derived stem cells treatment demonstrated favorable and sustainable therapeutic effect for Crohn's fistula. *Stem Cells (Dayton, Ohio)* 31:2575–2581
22. Cho YB, Park KJ, Yoon SN et al (2015) Long-term results of adipose-derived stem cell therapy for the treatment of Crohn's fistula. *Stem Cells Transl Med* 4:532–537
23. Molendijk I, Bonsing BA, Roelofs H et al (2015) Allogeneic bone marrow-derived mesenchymal stromal cells promote healing of refractory perianal fistulas in patients with Crohn's disease. *Gastroenterology* 149(918–27):e6
24. Panés J, Garcia-Olmo D, Van Assche G et al (2016) Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial. *Lancet (London, England)* 388:1281–1290
25. Park KJ, Ryoo SB, Kim JS et al (2016) Allogeneic adipose-derived stem cells for the treatment of perianal fistula in Crohn's disease: a pilot clinical trial. *Colorectal Dis* 18:468–476
26. Dietz AB, Dozois EJ, Fletcher JG et al (2017) Autologous mesenchymal stem cells, applied in a bioabsorbable matrix, for treatment of perianal fistulas in patients with Crohn's disease. *Gastroenterology* 153:59–62.e2
27. Panés J, García-Olmo D, Van Assche G et al (2018) Long-term efficacy and safety of stem cell therapy (Cx601) for complex perianal fistulas in patients with Crohn's disease. *Gastroenterology* 154:1334–1342.e4
28. Sinha R, Murphy P, Hawker P, Sanders S, Rajesh A, Verma R (2009) Role of MRI in Crohn's disease. *Clin Radiol* 64:341–352
29. Ren G, Zhao X, Wang Y et al (2012) CCR29-dependent recruitment of macrophages by tumor-educated mesenchymal stromal cells promotes tumor development and is mimicked by TNF α . *Cell Stem Cell* 11:812–824
30. Song B, Kim B, Choi SH et al (2014) Mesenchymal stromal cells promote tumor progression in fibrosarcoma and gastric cancer cells. *Korean J Pathol* 48:217–224
31. Dave M, Mehta K, Luther J, Baruah A, Dietz AB, Faubion WA Jr (2015) Mesenchymal stem cell therapy for inflammatory bowel disease: a systematic review and meta-analysis. *Inflamm Bowel Dis* 21:2696–2707
32. Cao Y, Ding Z, Han C, Shi H, Cui L, Lin R (2017) Efficacy of mesenchymal stromal cells for fistula treatment of crohn's disease: a systematic review and meta-analysis. *Dig Dis Sci* 62:851–860
33. Qiu Y, Li MY, Feng T et al (2017) Systematic review with meta-analysis: the efficacy and safety of stem cell therapy for Crohn's disease. *Stem Cell Res Ther* 8:136
34. Lightner AL, Wang Z, Zubair AC, Dozois EJ (2018) A systematic review and meta-analysis of mesenchymal stem cell injections for the treatment of perianal Crohn's disease: progress made and future directions. *Dis Colon Rectum* 61:629–640
35. Choi S, Jeon BG, Chae G, Lee SJ (2019) The clinical efficacy of stem cell therapy for complex perianal fistulas: a meta-analysis. *Tech Coloproctol*. <https://doi.org/10.1007/s10151-019-01994-z>
36. Wagner W, Bork S, Horn P et al (2009) Aging and replicative senescence have related effects on human stem and progenitor cells. *PLoS One* 4:e58846
37. Ivanova-Todorova E, Bochev I, Mourdjeva M et al (2009) Adipose tissue-derived mesenchymal stem cells are more potent suppressors of dendritic cells differentiation compared to bone marrow-derived mesenchymal stem cells. *Immunol Lett* 126:37–42
38. Duijvestein M, Molendijk I, Roelofs H et al (2011) Mesenchymal stromal cell function is not affected by drugs used in the treatment of inflammatory bowel disease. *Cytotherapy* 13:1066–1073

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