

REVIEW

Open Access



Efficacy of stem cells therapy for Crohn's fistula: a meta-analysis and systematic review

Yantian Cao¹ , Qi Su², Bangjie Zhang¹, Fangfang Shen³ and Shaoshan Li^{2*}

Abstract

Background: Fistulas have puzzled us all the time and stem cell therapy for it is still in its infancy. We conducted a meta-analysis and systematic review to evaluate the efficacy of stem cells and its potential mechanisms in the management of Crohn's fistula.

Methods: Electronic databases were searched comprehensively for studies reporting the efficacy and safety of stem cells in patients with any form of Crohn's fistula. A random-effects model was used, and all outcomes were calculated by SPSS 24.0.

Results: Twenty-nine articles with 1252 patients were included. It showed that stem cell group had a higher rate of fistula healing compared to placebo group in patients of Crohn's fistula (61.75% vs 40.46%, OR 2.21, 95% CI 1.19 to 4.11, $P < 0.05$). 3×10^7 cells/mL stem cell (SC) group had an advantage in fistula healing rate with 71.0% compared to other doses group of stem cells (RR 1.3, 95% CI 0.76 to 2.22). And the healing rates of patients with perianal and transsphincteric fistulas (77.95%, 76.41%) were higher than those with rectovaginal fistulas. It was an amazing phenomenon that CDAI and PDAI scores occurred an obviously transient rise with the use of stem cells after 1 month (both of $P < 0.05$), while they returned to the baseline level by giving stem cells 3 months later. Furthermore, the incidence rate of treatment-related adverse events in the stem cell group was significantly lower than in the placebo group (RR 0.58, 95% CI 0.30 to 1.14).

Conclusions: Our study has highlighted that stem cells was a promising method in the treatment of Crohn's fistula based on its higher efficacy and lower incidence of adverse events, especially ADSCs and Cx601. While it also needs more clinical and pre-clinical studies to strengthen evidences in the future.

Keywords: Stem cells, Crohn's fistula, Healing rate, Treatment-related adverse events

Introduction

Crohn's disease (CD) is a chronic, idiopathic inflammation of the whole gastrointestinal tract [1–3]. One of its common and baffling complications is fistula, an abnormal, tunnel-like connection between bowel and nearby epithelial surfaces [4, 5]. And its incidence rate is up to

17 to 50% among CD patients in worldwide [6], in which, 18 to 50% of patients with Crohn's fistula require surgical removal treatment according to population-based studies [7, 8]. And it is also characterized by facing a notoriously difficult surgical challenge of surgeons' skills due to its high recurrence [9]. Moreover, since patients with Crohn's fistula have suffered persistent fecal or urinary seepage, pain, and infection, Crohn's fistula brought people severe and diverse somatic and social issues, such as sleep disturbance, sexual dysfunction, and

* Correspondence: shaoshanlioo1@sina.com

²Department of General surgery, The Third Affiliated Hospital of Xinxiang Medical University, Hua Lan Avenue, Xinxiang 453003, Henan Province, China
Full list of author information is available at the end of the article



© The Author(s). 2021 **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

bad personal hygiene which reduced the quality of life ultimately [10–12]. Previous studies have shown that impaired local immune system, mucosal and transmural inflammation, luminal bacteria disorder, and hereditary susceptibility might play important roles [13–17]. Yet the specific mechanism is uncertain.

According to the high incidence, mortality, and disability rate, a large amount of researches about treatment of Crohn's fistula are on the way. The current conventional clinical managements include medications, endoscopic therapy, and surgery. Medications mainly include conventional anti-inflammatory agents such as 5-aminosalicylic acids (5-ASA) and systemic/local corticosteroids, as well as immunomodulators (azathioprine, 6-mercaptopurine (6-MP), cyclosporine, methotrexate, and anti-tumor necrosis factors (TNFs)) [18, 19]. Unfortunately, they have low rates of fistula closure like antibiotics of 21–48%, thiopurines of 20–40%, and infliximab of 23% [11] and have a recurrence rate of about 40.9% [20]. What is more, taking these drugs could lead to various adverse effects accompanied by lower compliance of patients [21–23]. With the development of diversities of endoscopy, they aimed at finding lesions and carrying drugs to the target in the patients of Crohn's fistula [24–27]. Its functions are limited. By comparison, surgical removal or drainage of fistulas is the mainstay option to eliminate them at present [28, 29]. But its incidence of postoperative complications was up to 50%, supported by Patil and Cross [30]. And a multicenter trial reported that improvement of inflammatory bowel disease questionnaire (IBDQ) and the MOS item short form health survey score (SF-36) had no difference in the laparoscopic ileocecal resection group of fistula and in the infliximab group (178.10 vs 172.00, 112.10 vs 106.50, $P > 0.05$, [31]). There was no doubt that combination of medical and surgical treatment had been superior to single medicine treatment in fistula closure (53% vs 43%, $P < 0.05$) [16].

Stem cell therapy (SCT) has emerged as a novel significant approach to improve the clinical remission and response in a number of inflammatory diseases and tissue regeneration due to its properties of immunoregulatory and multiple differentiation potential by releasing various mediators, including immunosuppressive molecules, growth factors, exosomes, chemokines, complement components, and multiple metabolites [32–34]. As the American Society of Colon and Rectal Surgeons said, the goals of Crohn's fistula management are alleviation of symptoms by eradication of the fistula, prevention of recurrence and preservation of sphincter integrity and continence [35]. Subsequently, SCT is envisaged as an effective alternative to patients of Crohn's fistula. Some animal studies had already demonstrated that bone marrow-derived stem cells (BMSCs) were able to repair injured intestinal mucosa through downregulating the

immune function of T lymphocytes [36]. Meanwhile, García-Olmo et al. conducted a phase I clinical trial in 2005, showing that the fistula healing rate (HR) reached to 60% with the use of adipose-derived mesenchymal stem cells (ADSCs) [37]. Since then, the application of ADSCs in Crohn's fistula has been explored increasingly due to its advantages of easy acquisition, low cost-saving of adipose tissue, and minimal invasion [32, 38]. Our previous review also reported that approximately 62.52% of patients with Crohn's fistula achieved complete clinical remission by using stem cells [39]. Until 2016, the study of Panés et al. (a randomized, double-blind, parallel-group, placebo-controlled trial) demonstrated that local allogeneic darvadstrocel (Cx601, Alofisel, Takeda) administration could be an effective and safe treatment for complex perianal fistulas in patients with Crohn's disease. Expanded allogeneic adipose-derived mesenchymal stem cells had been a novel, minimally invasive, well-tolerated, and alternative option in the treatment of Crohn's fistula in 2018, he said. To facilitate expanded development and application of stem cells in Crohn's disease, we have made great progress over the past 20 years, while it is still short of reliable assessment about stem cells (SCs) in the process of fistula healing in clinic, our objective is to redefine the role of stem cells and elaborate the mechanisms after SCT in patients of Crohn's fistula in this review.

Material and methods

Search strategy

We identified relevant studies by performing a comprehensive search of Pubmed and other databases (Cochrane Library and Embase) from the June 2005 to August 2020. The search was limited to clinical studies published in the English language. The search strategy was applied as below (all fields): (“mesenchymal stromal cells” OR “mesenchymal stem cells” OR “stem cells” OR “stroma cells”) AND (“fistula”) AND (“inflammatory bowel disease” OR “Crohn's disease”), and any appropriate abbreviations.

Study selection

All study selections were conducted by two reviewers independently without any discrepancy. Finally, they satisfied the following inclusion criteria: (1) clinical trials, (2) randomized controlled trials (RCTs) or non-randomized experimental studies (cohorts), (3) articles in English with full texts, (4) established diagnosis of CD or inflammatory bowel disease (IBD), (5) applied stem cells treatment for fistula, and (6) efficacy and/or adverse events were reported. Exclusion criteria were as follows: (1) repeated studies, (2) case reports, (3) letters or/and comments, (4) reviews or/and meta-analysis, (5) related with the pregnancy patients, and (6) non-English language articles.

Data extraction and assessment of study quality

Eligible articles were reviewed, the data were extracted and checked, and these were performed by two reviewers in a blind manner. And any disagreement in this process was resolved by discussion. Assessment of the quality of each study included was performed using Newcastle Ottawa Quality Assessment Scale (NOS), which involves 9 items for clinical trials [40]. All answers generated the final scores for each study. A high-quality article scores 5–9 [41].

Statistical analysis

We evaluated the degree of heterogeneity between studies using inconsistency index (I^2). Generally, the values of I^2 equal to 25%, 50%, and 75% were considered to indicate low, moderate, and high heterogeneity, respectively [42]. We adopted a random-effects model in our analysis. Statistical meta-analysis was performed in Review Manager 5.3 (Cochrane Collaboration, Oxford, UK) to generate forest plots of pooled risk ratio (RR), odds ratio (OR) and mean with 95% confidence intervals (CIs), and SPSS 24.0 was used to assess the efficacy (fistula healing rate) and treatment-related adverse events (TRAEs). Subgroup data was analyzed using the Chi-square test at the subgroup analysis (dose of stem cells, TRAEs), and $P < 0.05$ was considered statistically significant. Prism 6.0 was used for drawing pictures. Fistula healing, scores of Crohn disease activity index (CDAI), perianal disease activity index (PDAI), inflammatory bowel disease questionnaire (IBDQ), and level of C-reactive protein (CRP) were assessed during the follow-up.

Results

Search results and study characters

A total of 901 articles were researched by our search strategy, of which 29 were included and a total of 1252 fistula patients were enrolled in our analysis by the eligibility criteria (Fig. 1), and study characteristics were shown in Table 1. There were 22 studies applied with ADSCs (four from Alofisel, Cx601, 16 from homemade cultures, one from Cx401, and one without a clear source), six with BMSCs and one with adipose tissue. The phases II–III studies accounted for approximately 44.83% (13/29) and phase I for 20.69% (6/29). Only 12 in 29 studies were RCTs: comparing applied with stem cells to placebo (e.g., Fibrin glue) [43–52], and comparing patients of Crohn's fistula to those not with CD [43, 44, 53, 54]. The remaining received SCT with no control group. Eighteen studies used autologous stem cells [37, 44–47, 50, 55–66], seven with allogeneic stem cells [48, 49, 51, 52, 67–69], and both of them were used in other two studies [53, 54].

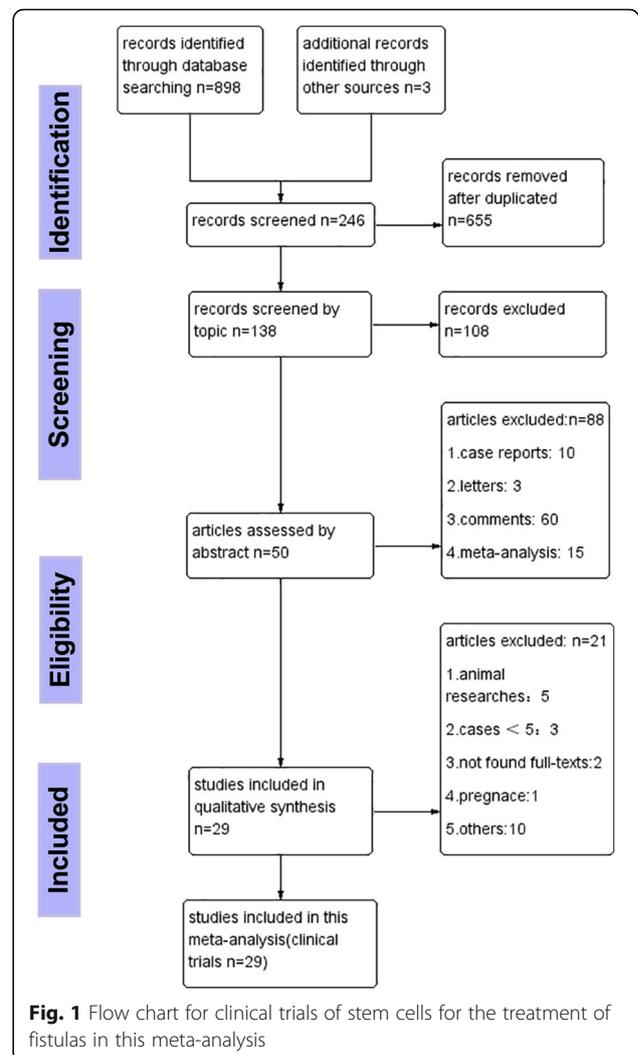


Fig. 1 Flow chart for clinical trials of stem cells for the treatment of fistulas in this meta-analysis

Quality assessment

The qualities of included studies were assessed by using the NOS, and we analyzed 29 clinical studies enrolled and finally had a total score of 175 with a mean of 6 and a range of 3 to 9 for each study (Supplement Table 1). In total, the quality of clinical trials reached the “high-quality study” level.

Primary outcomes

Efficacy of stem cells therapy for Crohn's fistulas

Four of 12 RCTs reported fistula healing in Crohn's fistula and un-CD fistula [43, 44, 53, 54]. Seven reported fistula healing administrated with stem cells and placebo (such as fibrin glue) [43, 44, 47–51]. The random-effects model was used to assess the differences in healing rate (HR) between the experiment group (Crohn's fistulas, stem cell group) and control group (un-CD fistulas, placebo group). Regardless of the dose of stem cells injected and route of administration, the HRs of fistula in

Table 1 Characteristics of clinical studies included

Author	Year of publication	Phase	Age (M ± SD)	Sex (M: F)	Number of case included	Source of cell	Type of cell	Dose of cell injected	Donor	Follow-up*	Outcomes
Both of CD and un-CD fistula											
García-Olmo et al. [43]	2009	II	43.33 ± 9.9	24: 25	CD, 14 (ASC, 7; FG, 7); unCD, 35 (ASC, 17; FG, 18)	AT, Cx401	ADSC	1 × 10 ⁷ cells/ml	NR	7 M	FH, SF-12, SAE
Guadalajara et al. [44]	2012	II	42.6 ± 10.9	24: 25	CD, 20 (ASC+FG, 17; FG, 3); unCD, 29 (ASC+FG, 7; FG, 22)	Liposuction, NR	ADSC	NR	Autologous	5 Y	FH, MRI, SAE
García-Olmo et al. [53]	2015	I–II–III	49	8:2	CD, 3; un-CD, 7	AT, homemade	ADSC/SVF	1–3 × 10 ⁷	Autologous/allogeneic	1 Y	FH, Wexner scores, SAE
Herreros et al. [54]	2019	NR	45	24: 21	CD, 18; un-CD, 24	NR, homemade	ASC/SVF	2 × 10 ⁶ cells/ml	Autologous/allogeneic	1 Y	FH, AE, SAE
Only un-CD fistula											
Herreros et al. [45]	2012	III	49.78	NR	ASC, 64; ASC+FG, 60; FG, 59	AT, homemade	ADSC	First, 2 × 10 ⁷ cells/ml; second, 4 × 10 ⁷ cells/ml	Autologous	1 Y	SF-36Q, Wexner score 22, FCS, SAE
Choi et al. [55]	2017	II	37.9	13:0	1 × 10 ⁷ cells/mL, 5; 2 × 10 ⁷ cells/mL, 8	AT, homemade	ADSC	2 × 10 ⁷ or 4 × 10 ⁷ cells/ml	Autologous	6 M	FH, Wexner scale, SAEs
Dozois et al. [56]	2019	I	39.8	7:8	15	AT, homemade	AD- MSC	NR	Autologous	6 M	FH, MRI, SAE
Topal et al. [57]	2019	NR	47 ± 13.1	8:2	10	AT, homemade	ADSC	NR	Autologous	9 M	PE, SAE
García-Arranz et al. [46]	2020	III	50 ± 10	30: 14	10 × 10 ⁷ ASC+FG, 23; FG, 21	NR, NR	ADSC	10 × 10 ⁷ cells/ml	Autologous	2 Y	PE, MRI, SF-12, Wexner incontinence score, SAE
Only CD fistula											
García-Olmo et al. [37]	2005	I	35.1 ± 2.4	2:3	5	AT, homemade	AD- MSC	1–3 × 10 ⁷ cells/ml	Autologous	30 M	FH, MRI, SAE
Ciccocioppo et al. [58]	2011	NR	32	8:4	12	BM, homemade	BM- MSC	NR	Autologous	1 Y	CDAI; PDAI; MRI; endoscopy; FoxP3; T cell; IL-2, 5, 10, and 12; IFN; TNF-α; SAE
Cho et al. [59]	2013	II	26. ± 6.0	4:6	1 × 10 ⁷ cells/ml, 3; 2 × 10 ⁷ cells/ml, 4; 4 × 10 ⁷ cells/ml, 3	AT, homemade	ADSC	1/2/4 × 10 ⁷ cells/ml	Autologous	8 M	FH, CD4/CD8 ratio, SAE
de la Portilla et al. [60]	2013	I/IIa	36 ± 9.0	11: 13	2 × 10 ⁷ cells/ml, 9; 6 × 10 ⁷ cells/ml, 15	AT, homemade	ADSC	First, 2 × 10 ⁷ cells/ml; second, 4 × 10 ⁷ cells/ml	Autologous	6 M	MRI, PDAI, CDAI, SAE
Lee et al. [61]	2013	II	26.2 ± 5.4	30: 13	42	AT, homemade	ADSC	First, 15.8 × 10 ⁷ ; second, 19.1 ×	Autologous	1 Y	FH, SAE

Table 1 Characteristics of clinical studies included (*Continued*)

Author	Year of publication	Phase	Age (M ± SD)	Sex (M: F)	Number of case included	Source of cell	Type of cell	Dose of cell injected	Donor	Follow-up*	Outcomes
Molendijk et al. [47]	2015	NR	37.3	12:9	1 × 10 ⁷ cells/ml, 5; 3 × 10 ⁷ cells/ml, 5; 9 × 10 ⁷ cells/ml, 5; placebo, 6	BM, homemade	ADSC	1/3/9 × 10 ⁷ cells/ml	Autologous	2 Y	FH, MRI, PDAI, CDAI, IBDQ, CDEIS, SES-CD, SF-36, CRP, IL-8, IL-1β, IL-6 and 10, TNF, IL-12p70, SAE
Ciccocioppo et al. [62]	2015	NR	39.25 ± 14.32	5:3	8	BM, homemade	BM- MSC	NR	Autologous	6 Y	MRI, CDAI, PDAI, SAE
Cho et al. [63]	2015	II	26.2 ± 5.5	28:13	41	AT, homemade	ADSC	3 × 10 ⁷ cells/ml	Autologous	2 Y	FH, AE, SAE
Park et al. [67]	2016	NR	32.17 ± 7.96	4:2	1 × 10 ⁷ cells/ml, 3; 3 × 10 ⁷ cells/ml, 3	AT, homemade	ADSC	1 × 10 ⁷ cells/ml, 3 × 10 ⁷ cells/ml	Allogeneic	6 M	FH, MRI, CD4/CD8 ratio, SAE
Panés et al. [48]	2016	III	38	116:96	ASC, 107; placebo, 105	AT, alofisel	AD- MSC	12 × 10 ⁷ cells	Allogeneic	6 M	MRI, PDAI, CDAI, IBDQ, van Assche score, IgG AE, SAE
García-Arranz et al. [68]	2016	I-IIa	35	NR	10	AT, homemade	ADSC	First, 2 × 10 ⁷ cells; second, 4 × 10 ⁷ cells	Allogeneic	4.5 M	QoL, SF-36, fecal incontinence, severity index
Dietz et al. [64]	2017	I	35 ± 14.21	6:6	12	BM, homemade	MSC	2 × 10 ⁷ cells/ml	Autologous	6 M	MRI, Van Assche score, SAE
Scott [69]	2018	III	38	NR	24	AT, alofisel	ADSC	12 × 10 ⁷ cells	Allogeneic	13 M	IL-6, 12, and 10; TNF-α; TGF-β; PDAI; CDAI; SAE
Panés et al. [49]	2018	III	38.3	114:98	12 × 10 ⁷ cells, 107; placebo, 105	AT, alofisel	ADSC	12 × 10 ⁷ cells	Allogeneic	13 M	IBDQ, PDAI, CDAI, TNF, MRI, TEAE
Wainstein et al. [70]	2018	NR	36	2:7	9	AT, homemade	ADSC	NR	Autologous	37 M	IBDQ, PDAI, SAE
Avivar-Valderas et al. [52]	2019	NR	NR	NR	ASC 58; placebo 42	AT, Alofisel	AD- MSC	NR	Allogeneic	13 M	DSA; HLA-I; CRPs; CD55, 46, and 59; AE, SAE
Dige et al. [66]	2019	NR	NR	6:15	21	AT, homemade	AT	NR	Autologous	6 M	FH, complications
Zhou et al. [50]	2020	NR	28.86 ± 10.13	21:1	ADSC, 11; placebo, 11	AT, homemade	ADSC	5 × 10 ⁶ cells/ml	NR	1 Y	FH, MRI, ultrasonography, CDAI, PDAI, IBDQ, VAS, Wexner score, CRP, ESR, FC, SAE
Barnhoorn et al. [51]	2020	NR	42	8:6	1 × 10 ⁷ cells/ml, 5; 3 × 10 ⁷ cells/ml, 5; 9 × 10 ⁷ cells/ml, 5; placebo, 6	BM homemade	BM- MSC	1/3/9 × 10 ⁷ cells/ml	Allogeneic	4 Y	FH, HLA, MRI, rectoscopy, PDAI, CDAI, Vaizey, QoL, SF36, IBDQ, SAE
Lightner et al. [65]	2020	NR	49	0:5	5	BM, homemade	MSC	NR	Autologous	6 M	FH, MRI, AE, SAE

*Y = years and M = months

Abbreviations: NR not report, FG fibrin glue, AT adipose tissue, BM bone marrow, ADSC adipose-derived stem cells, SVF stromal vascular fraction, MSC mesenchymal stem cell, BM-MSK bone marrow-derived mesenchymal stromal cells, FH fistula healing, PE physical examination, QoF quality of life, MRI magnetic resonance imaging, FCS fistula complexity score, CDAI Crohn's disease activity index, PDAI perianal disease activity index, CDEIS Crohn's disease endoscopic index of severity, SES-CD simplified endoscopic activity score, CRP C-reactive protein, TGF-β transforming growth factor-β, DSA donor-specific antibodies, VSA pain scores with visual analog score, ESR erythrocyte sedimentation rate, FC fecal calprotectin, GF growth factor, BLI bioluminescence, TEAEs treatment-emergent adverse events

patients with CD and without CD were 58.71% and 58.72%, respectively ($I^2 = 0\%$, RR 0.00, 95% CI - 0.20 to 0.20, $P > 0.05$) (Fig. 2a). Moreover, patients with Crohn's fistula in the stem cell group had a higher HR of 61.75% than in the placebo group (40.46%). There was a significant statistical difference ($I^2 = 35\%$, RR 2.21, 95% CI 1.19 to 4.11, $P = 0.01$) (Fig. 2b). As for the source of stem cells, HR of Cx601 administration in patients of Crohn's fistula was about 61.02%, which was higher than of ASCs from homemade cultures (51.43%, $P < 0.05$).

The doses of stem cells injected

There were four clinical trials involving the different doses of SCT [47, 51, 59, 67], and a total of 31 patients of Crohn's fistula received the different doses of stem cells. They were divided into five subgroups: 1×10^7 cells/mL, 2×10^7 cells/mL, 3×10^7 cells/mL, 4×10^7 cells/mL, and 9×10^7 cells/mL groups, respectively. The patients injected with the dose of 1×10^7 cells/mL group were defined as the control group, and others were experimental groups. As Fig. 3a showed, only one study was treated with stem cells of doses of 2×10^7 cells/mL ($n = 7$) and 4×10^7 cells/mL ($n = 6$), which was lack of completely valid data [59], and three studies with stem cells of dose of 3×10^7 cells/mL ($n = 24$, [47, 51, 67]), two with 9×10^7 cells/mL ($n = 19$, [47, 51]). The dose of 3×10^7 cells/mL group had a stronger advantage in Crohn's fistula healing compared to 1×10^7 cells/mL group ($I^2 = 0.00\%$, OR 1.30, 95% CI 0.76 to 2.22), but the dose of 9×10^7 cells/mL group was defeated by the 1×10^7 cells/mL group in terms of fistula healing ($I^2 = 0.00\%$, OR 0.35, 95% CI 0.09 to 1.39). The HRs of patients with Crohn's fistula in 1×10^7 cells/mL, 2×10^7 cells/mL, 3×10^7 cells/mL, 4×10^7 cells/mL, and 9×10^7 cells/mL group were 50.60%, 69.50%, 71.00%, 33.00%, and 20.00%,

respectively (showed in Fig. 3b). Compared to 4×10^7 cells/mL and 9×10^7 cells/mL groups, there were statistically significant differences in 2×10^7 cells/mL and 3×10^7 cells/mL groups, respectively (both of $P < 0.05$). Overall, the dose of 3×10^7 cells/mL stem cells had a higher superiority in patients of Crohn's fistula.

The types of Crohn's fistula

Nine of 29 studies reported HRs based on fistula types with a total of 99 fistulas [37, 43, 44, 54, 64, 66–68, 70]. They mainly comprised perianal, transsphincteric, and rectovaginal fistulas. Our analysis involved in perianal fistula with seven studies, transsphincteric fistula with three studies, and rectovaginal fistula with seven. As Fig. 3c showed, the patients with perianal and transsphincteric fistulas had more apparent HRs compared to those with rectovaginal fistulas (77.95%, 76.41% vs. 27.18%, $P < 0.01$).

Secondary outcomes

Related scores and indicator assessment

Most of clinical studies reported the detailed changes of scores and lab indicators, such as CDAI, PDAI, IBDQ, and CRP. Of them, four studies reported variation of CDAI after administrating stem cells [47, 49, 58, 60], seven articles were referred to changes of PDAI [47–50, 58, 60, 70], five to scores of IBDQ [47, 49–51, 70], and two to the level of CRP [47, 50]. For scores of CDAI, 1 month later, it occurred a transient rise among patients with the treatment of stem cell (295.00 vs. 132.10, $P < 0.05$), while 3 months later, it returned to the baseline score and last to endpoint (295.00 vs. 111.14, $P < 0.05$) (Fig. 4a). It had a mild difference in the variation of PDAI score—there were two rising tendency after 1 month and 12 months by SCT, respectively, and it downed to 3.54 and 3.31 until 3 and 6 months after giving SCs. There was a statistical significant difference between pre-stem cells and post-stem cells ($P < 0.05$) (Fig. 4b). Besides that, scores of IBDQ did not increase until 6 months later by SCT and reached a peak at 12 months with no significance difference (88.85 ± 69.96 vs. 181.95 ± 8.27 , $P > 0.05$). Moreover, after stem cells therapy, the level of CRP (nmol/l) showed continuous drops (baseline vs. 6 months, 11.3 ± 13.23 vs. 4.5 ± 4.7 , $P < 0.05$). These details could be found in Figs. 4a and b.

TRAEs analysis

All of studies showed the adverse events (AEs) and severe adverse events (SAEs). However, in terms of TRAEs, the details and accurate data are only reported in three RCTs [48–50] and eight cohort studies. Figure 5 showed the difference of the incidence rate of TRAEs between the stem cell group and placebo group, patients in the stem cell group had an advantage over in placebo group ($I^2 = 59\%$, RR 0.58, 95% CI 0.30 to 1.14). In cohort

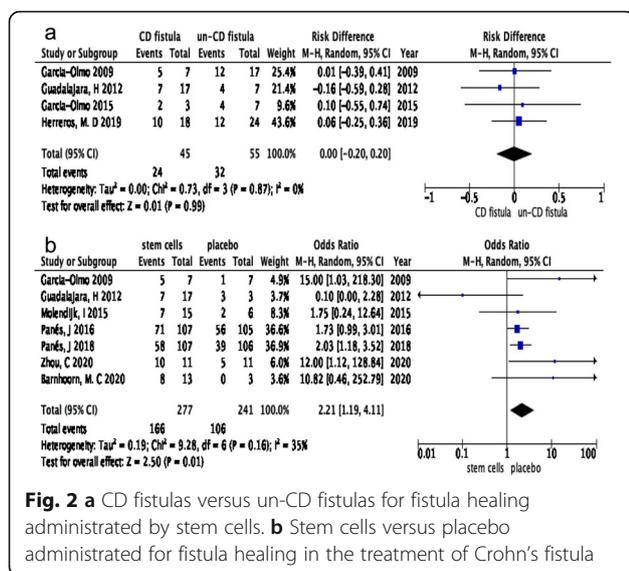
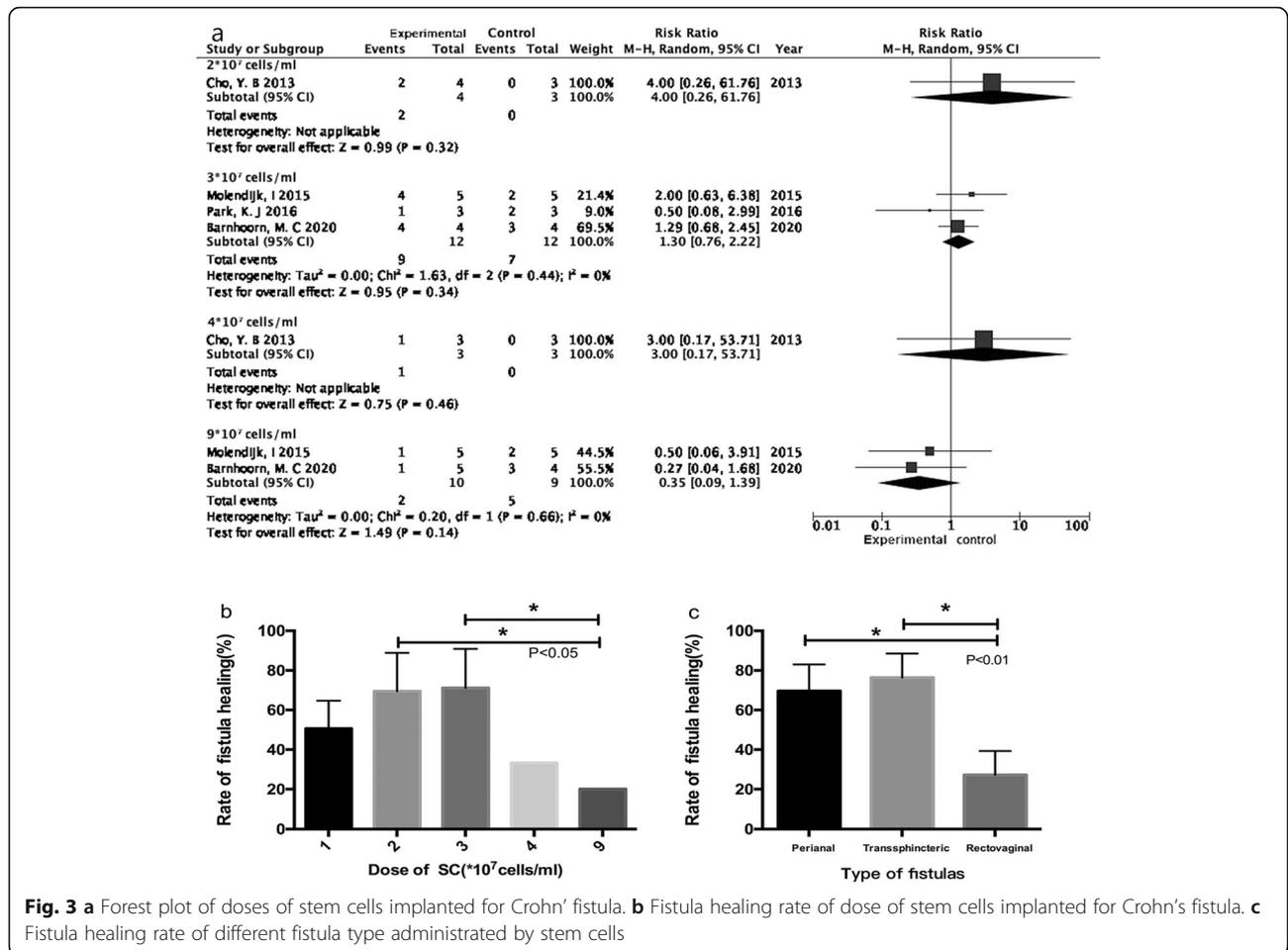


Fig. 2 a CD fistulas versus un-CD fistulas for fistula healing administrated by stem cells. **b** Stem cells versus placebo administrated for fistula healing in the treatment of Crohn's fistula



studies, only studies by de la Portilla et al. and Scott et al. [60, 69] reported five TRAEs.

Discussion

To our knowledge, this review summarized that SCT had a higher efficacy and lower incidence of adverse events in patients with Crohn's fistula compared with conventional therapies. We revealed that the rate of fistula healing ranged from 58 to 62% after administrating SCs in patients of Crohn's fistula, which was similar to

our previous results with a clinical remission of 62.52% [39]. What is more, Cx601 (darvadstrocel) administration in patients of Crohn's fistula had a higher HR of 61.02% compared to homemade cultures, it had strongly evidenced that it was necessary and important for a systematic, maturing and professional protocol in clinical work. Our analysis also found, whether patients suffered Crohn's fistula or not (un-CD fistula), the HR had no statistically significant difference (58.71% vs. 58.72%). Considering the properties of immunoregulatory, anti-

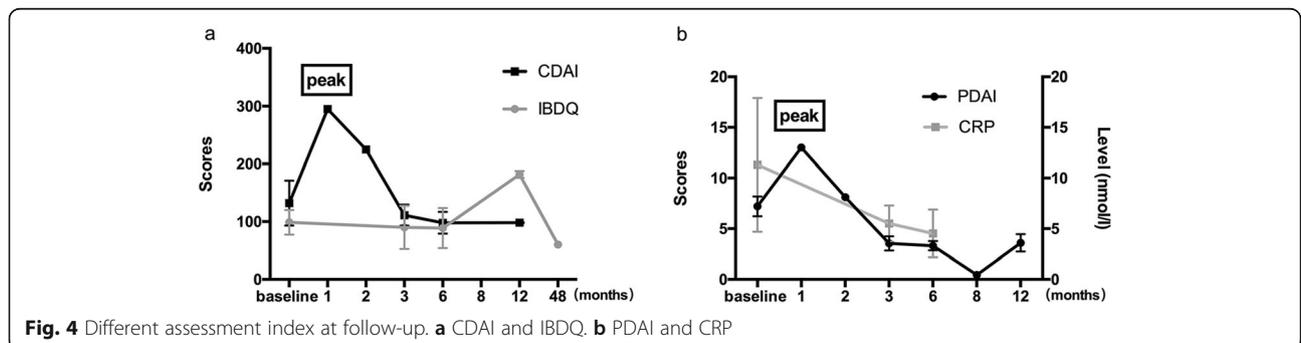


Fig. 4 Different assessment index at follow-up. **a** CDAI and IBDQ. **b** PDAI and CRP

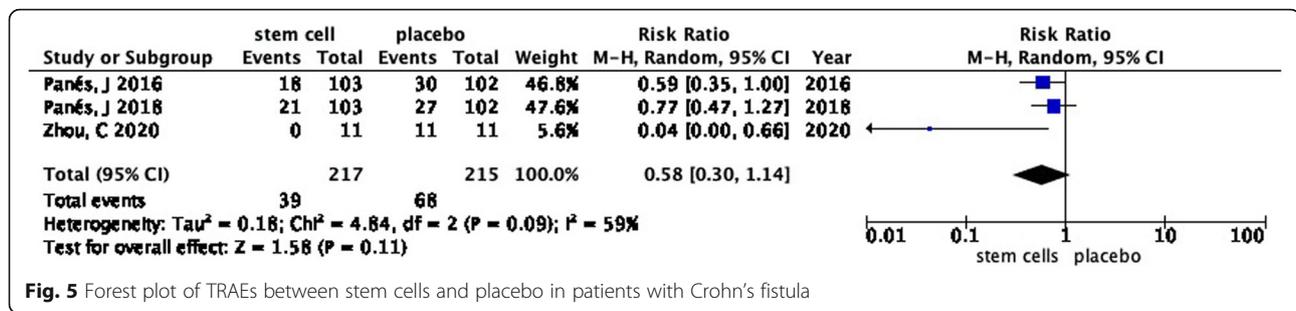


Fig. 5 Forest plot of TRAEs between stem cells and placebo in patients with Crohn's fistula

inflammatory, and multipotential differentiation of stem cells [33, 34, 71–73], which one dominated the important position and what the potential mechanism was? The possible explanations were presented as follows: primarily, SCs could alter immune reconstitution by stimulating de novo generation of an altered T cell repertoire and eliminating aberrant clones, it then would replace fistulas [74]. Secondly, SCs could release various mediators and multiple metabolites, such as growth factors, exosomes, and chemokines, to repair injured tissue and promote its regeneration by strengthening differentiation [75]. Plus, the study by Clevers et al. also had referred to “niche” of stem cells, and it was able to promote self-organization of multiple mammalian tissues by the self-renewal factors of Wnt signaling [76]. Conversely, stem cells' fate was influenced by various growth factors to differentiate into normal tissues [77]. Obviously, the latter match more with roles of stem cells in this process among patients of Crohn's fistula and un-CD fistula. It was, therefore, worth supposing that stem cells might work in patients of Crohn's fistula and un-CD fistula by means of multi-directional differentiation to achieve tissue repair, rather than of immune reconstitution only.

With respect to the doses of SCT in patients of Crohn's fistula in our review, data on the use of stem cells at different doses was limited. And a variety of articles consistently demonstrated that an optimal dose was the key point in the protocol of stem cells for Crohn's fistula and was a focused problem for a long time in clinical and pre-clinical studies. In this study, only two clinical studies by Molendijk et al. and Barnhoorn et al. [47, 51] randomly assigned CD patients to three groups based on doses of stem cells: 1×10^7 cells/mL, 3×10^7 cells/mL, and 9×10^7 cells/mL, and they demonstrated that 3×10^7 cells/mL group had the highest HR. Meanwhile, the study of Scott reported that a single dose of 120 million stem cells (Darvadstrocel) administered into the perianal fistulas tissue was significantly more effective than placebo (saline) with a clinical remission of more than 50% [69]. Additionally, our published articles had put forward that the best range of dose of stem cells in Crohn's fistula was $2\text{--}4 \times 10^7$ cells/mL with a HR of up to 80.07% [39]. Surprisingly, we found that stem cells

at the dose of 3×10^7 cells/mL had the highest HR of 71.00% compared to other doses of cells in this meta-analysis, which was beneficial for improving the efficacy in Crohn's fistula at a large degree and narrowed down the optimal dose of SCs further.

Given the classification of fistulas, they could be divided into simple and complex fistulas based on American Gastroenterological Association classification system [78, 79], as well as into intersphincteric, transsphincteric, extrasphincteric, or suprasphincteric fistulas by anatomy [80]. In our study, it referred mostly to perianal, rectovaginal, intersphincteric, and transsphincteric fistulas. Only nine of them reported the fistula healing rate based on the fistula types. Surprisingly, we noticed that patients of perianal and transsphincteric fistulas had a better reaction to SC implantation than of rectovaginal fistula with the efficacy of 77.95% and 76.41%, respectively. But the reason was not lucid. Among patients of perianal fistulas, the inflammation was extremely severe due to roles of intestinal bacterial and communication of excrement and perianal skin surface to trigger an immune response and cytokine production [43, 81]. As the study by Cellerix et al. supported, SCs could be stimulated by relatively high concentrations of pro-inflammatory cytokines (IFN- γ) produced by fistula lesions, and expressed indoleamine 2, 3-dioxygenase (IDO) of metabolizing tryptophan to kynurenine, which had an anti-inflammatory effect. So, let us make a hypothesis that in the relatively severe inflammatory microenvironment of perianal area, SCs play a remarkable role of anti-inflammatory, which was consistent with what we discussed previously (the “Discussion” section, paragraph 1, [82, 83]). Meanwhile, the second reason, perianal and transsphincteric fistulas themselves were also believed to own a relatively dry and comfortable local anatomical circumstance, which contribute to renovate impaired tissue being different from rectovaginal fistulas [54].

CDAI was defined to evaluate the efficacy and severity of Crohn's disease, as well as PDAI [84]. In 2017, our study already showed that there was a different clinical remission between patients of CDAI baseline >150 group and <150 group by contrasting the change of level of CDAI after SCT [39]. While it did not refer to

the change of CDAI at each time point—we solved it in this review. Coincidentally, we found a transient rise at 1 month extensively and reduced to a level below the baseline at 3 months after injecting SCs. Which of the mechanisms were playing significant roles in it? An animal experiment might have mirrored our results: once stem cells were injected into fistula walls among rats of Crohn's fistula, effective stem cell mass was reduced from inception, and the detectable cells represented only 8.87% of their initial amount to take action at the first 30 days via monitoring the dynamics of bioluminescence (BLI) [85]. Moreover, the study of TiGenix et al. [86] had confirmed that ADSCs were present in the rectum and jejunum for ≥ 14 days and undetectable after 3 or 6 months in any of the tissues. Taken together, we can hypothesize that at the period of 1 month after SCT, up to 90% of stem cells vanished and destroyed by peoples' immune clearance themselves; after 3 months, the remaining SCs (about 8%) were differentiated into functional epithelial cells or stromal cells; meanwhile, the phases of immune tolerance and reconstruction started up [74]. The defined and powerful mechanisms need further pre-clinical and clinical studies.

Limitations

Several limitations of our meta-analysis should be acknowledged: (1) In terms of animal researches, they were small-sized and lacked detailed data about mucous healing; assessment index, such as IL-2, IL-6, IL-8, and IL-10; and the adverse events, which failed to analyze systematically. (2) In clinical trials, they did not report the results about immunohistochemistry and endoscopy in quantification. (3) Subgroup analysis was inadequate owing to the majority of studies included about cohort studies. (4) Given the limited follow-up among included studies, we failed to elaborate the recurrent after administering stem cells among CD patients. (5) We used random-effects model to account for the statistical heterogeneity conservatively in the pooled studies.

Conclusions

The utility of stem cells in patients of Crohn's disease is a potential way, but still in the very early stages, particularly in Crohn's fistula; the achievements are encouraging now but not comprehensive and systematic. Based on our review, SC treatment in treatment of Crohn's fistula has a higher efficacy (fistula healing rate), as well as a lower TRAEs compared to other options. And the optimal dose of 3×10^7 cells/mL SCs injected has been determined. While a gold standard is still not be identified, we are eager to do more basic and clinical studies performed to further ascertain protocol and break current dilemmas.

Supplementary Information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s13287-020-02095-7>.

Additional file 1.

Abbreviations

SCs: Stem cells; SCT: Stem cell therapy; RR: Risk ratios; OR: Odds ratio; CIs: Confidence intervals; ADSCs: Adipose-derived stem cells; RCTs: Random control trials; HR: Healing rate; CD: Crohn disease; IBD: Inflammatory bowel disease; 5-ASA: 5-Aminosalicylic acids; 6-MP: 6-Mercaptopurine; TNFs: Anti-tumor necrosis factors; SF-36: MOS item short from health survey score; BMSCs: Bone marrow-derived stem cells; NOS: Newcastle Ottawa Quality Assessment Scale; TRAEs: Treatment-related adverse events; CDAI: Crohn disease activity index; PDAI: Perianal disease activity index; IBDQ: Inflammatory bowel disease questionnaire; CRP: C-reactive protein; AEs: Adverse events; SAEs: Severe adverse events

Acknowledgements

Thanks a lot for the financial support by Henan Medical Science and Technology innovation talent in the study.

Authors' contributions

YC participated in the design of the study and drafted the manuscript. QS participated in the collection of study and performed the statistical analysis. BZ conceived of the study, and participated in its design and coordination and helped to draft and modify the manuscript. FS and SL revised the manuscript and polished the English language. All authors read and approved the final manuscript.

Funding

No.

Availability of data and materials

The data supporting the conclusions of this article are all online.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

Author details

¹Department of Gastroenterology, The Third Affiliated Hospital of Xinxiang Medical University, Hua Lan Avenue, Xinxiang 453003, Henan Province, China. ²Department of General surgery, The Third Affiliated Hospital of Xinxiang Medical University, Hua Lan Avenue, Xinxiang 453003, Henan Province, China. ³The Key Laboratory for Tumor Translational Medicine, The Third Affiliated Hospital of Xinxiang Medical University, Hua Lan Avenue, Xinxiang 453003, Henan Province, China.

Received: 9 November 2020 Accepted: 10 December 2020

Published online: 07 January 2021

References

- Kappelman MD, Rifas-Shiman SL, Kleinman K, Ollendorf D, Bousvaros A, Grand RJ, Finkelstein JA. The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States. *Clin Gastroenterol Hepatol.* 2007;5(12):1424–9.
- Sonnenberg A, Genta RM. Geographic distributions of microscopic colitis and inflammatory bowel disease in the United States. *Inflamm Bowel Dis.* 2012;18(12):2288–93.
- Loftus EV Jr. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. *Gastroenterology.* 2004;126(6):1504–17.
- Torres J, Mehandru S, Colombel JF, Peyrin-Biroulet L. Crohn's disease. *Lancet.* 2017;389(10080):1741–55.

5. Lee MJ, Parker CE, Taylor SR, Guizzetti L, Feagan BG, Lobo AJ, Jairath V. Efficacy of medical therapies for fistulizing Crohn's disease: systematic review and meta-analysis. *Clin Gastroenterol Hepatol*. 2018;16(12):1879–92.
6. Schwartz DA, Loftus EV Jr, Tremaine WJ, Panaccione R, Harmsen WS, Zinsmeister AR, Sandborn WJ. The natural history of fistulizing Crohn's disease in Olmsted County, Minnesota. *Gastroenterology*. 2002;122(4):875–80.
7. Bouguen G, Peyrin-Biroulet L. Surgery for adult Crohn's disease: what is the actual risk? *Gut*. 2011;60(9):1178–81.
8. Fumery M, Singh S, Dulai PS, Gower-Rousseau C, Peyrin-Biroulet L, Sandborn WJ. Natural history of adult ulcerative colitis in population-based cohorts: a systematic review. *Clin Gastroenterol Hepatol*. 2018;16(3):343–356.e343.
9. Torres J, Billioud V, Sachar DB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis as a progressive disease: the forgotten evidence. *Inflamm Bowel Dis*. 2012;18(7):1356–63.
10. Kotze PG, Shen B, Lightner A, Yamamoto T, Spinelli A, Ghosh S, Panaccione R. Modern management of perianal fistulas in Crohn's disease: future directions. *Gut*. 2018;67(6):1181–94.
11. Gecece KB, Bemelman W, Kamm MA, Stoker J, Khanna R, Ng SC, Panés J, van Assche G, Liu Z, Hart A, et al. A global consensus on the classification, diagnosis and multidisciplinary treatment of perianal fistulising Crohn's disease. *Gut*. 2014;63(9):1381–92.
12. Williet N, Sarter H, Gower-Rousseau C, Adrianjafy C, Olympie A, Buisson A, Beaugerie L, Peyrin-Biroulet L. Patient-reported outcomes in a French nationwide survey of inflammatory bowel disease patients. *J Crohns Colitis*. 2017;11(2):165–74.
13. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. *Nature*. 2011;474(7351):307–17.
14. Steel AW, Mela CM, Lindsay JO, Gazzard BG, Goodier MR. Increased proportion of CD16(+) NK cells in the colonic lamina propria of inflammatory bowel disease patients, but not after azathioprine treatment. *Aliment Pharmacol Ther*. 2011;33(1):115–26.
15. Ballester Ferré MP, Boscá-Watts MM, Mínguez Pérez M. Crohn's disease. *Med Clin (Barc)*. 2018;151(1):26–33.
16. Lopez N, Ramamoorthy S, Sandborn WJ. Recent advances in the management of perianal fistulizing Crohn's disease: lessons for the clinic. *Expert Rev Gastroenterology Hepatol*. 2019;13(6):563–77.
17. Haac BE, Palmateer NC, Seaton ME, Van YR, Fraser CM, Bafford AC. A distinct gut microbiota exists within Crohn's disease-related perianal fistulae. *J Surg Res*. 2019;242:118–28.
18. Feuerstein JD, Cheifetz AS. Crohn disease: epidemiology, diagnosis, and management. *Mayo Clin Proc*. 2017;92(7):1088–103.
19. Eustace GJ, Melmed GY. Therapy for Crohn's disease: a review of recent developments. *Curr Gastroenterol Rep*. 2018;20(5):19.
20. de Groof EJ, Sahami S, Lucas C, Ponsioen CY, Bemelman WA, Buskens CJ. Treatment of perianal fistula in Crohn's disease: a systematic review and meta-analysis comparing seton drainage and anti-tumour necrosis factor treatment. *Colorectal Dis*. 2016;18(7):667–75.
21. Ruemmele FM, Veres G, Kolho KL, Griffiths A, Levine A, Escher JC, Amil Dias J, Barabino A, Braegger CP, Bronsky J, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. *J Crohns Colitis*. 2014;8(10):1179–207.
22. Toruner M, Loftus EV Jr, Harmsen WS, Zinsmeister AR, Orenstein R, Sandborn WJ, Colombel JF, Egan LJ. Risk factors for opportunistic infections in patients with inflammatory bowel disease. *Gastroenterology*. 2008;134(4):929–36.
23. Ansari A, Patel N, Sanderson J, O'Donohue J, Duley JA, Florin TH. Low-dose azathioprine or mercaptopurine in combination with allopurinol can bypass many adverse drug reactions in patients with inflammatory bowel disease. *Aliment Pharmacol Ther*. 2010;31(6):640–7.
24. Fefferman DS, Farrell RJ. Endoscopy in inflammatory bowel disease: indications, surveillance, and use in clinical practice. *Clin Gastroenterol Hepatol*. 2005;3(1):11–24.
25. Leong RW, Ooi M, Corte C, Yau Y, Kermeen M, Katelaris PH, McDonald C, Ngu M. Full-spectrum endoscopy improves surveillance for dysplasia in patients with inflammatory bowel diseases. *Gastroenterology*. 2017;152(6):1337–1344.e1333.
26. Le L, Fung BM, Tabibian JH. Capsule endoscopy for refractory iron deficiency anemia in Crohn's disease: captivating pathology hybrid therapy. *Gastroenterology*. 2018;155(2):276–7.
27. Spiceland CM, Lodhia N. Endoscopy in inflammatory bowel disease: role in diagnosis, management, and treatment. *World J Gastroenterol*. 2018;24(35):4014–20.
28. Van Assche G, Dignass A, Panes J, Beaugerie L, Karagiannis J, Allez M, Ochsenkühn T, Orchard T, Rogler G, Louis E, et al. The second European evidence-based consensus on the diagnosis and management of Crohn's disease: definitions and diagnosis. *J Crohns Colitis*. 2010;4(1):7–27.
29. Lee JL. Factors affecting surgical treatment with infliximab therapy in perianal fistula with Crohn disease. *Ann Coloproctol*. 2019;35(5):225–6.
30. Patil SA, Cross RK. Medical versus surgical management of penetrating Crohn's disease: the current situation and future perspectives. *Expert Rev Gastroenterol Hepatol*. 2017;11(9):843–8.
31. Ponsioen CY, de Groof EJ, Eshuis EJ, Gardenbroek TJ, Bossuyt PMM, Hart A, Warusavitarnae J, Buskens CJ, van Bodegraven AA, Brink MA, et al. Laparoscopic ileocaecal resection versus infliximab for terminal ileitis in Crohn's disease: a randomised controlled, open-label, multicentre trial. *Lancet Gastroenterol Hepatol*. 2017;2(11):785–92.
32. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*. 2002;13(12):4279–95.
33. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, Lanino E, Sundberg B, Bernardo ME, Remberger M, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet*. 2008; 371(9624):1579–86.
34. Forbes GM, Sturm MJ, Leong RW, Sparrow MP, Segarajasingam D, Cummins AG, Phillips M, Herrmann RP. A phase 2 study of allogeneic mesenchymal stromal cells for luminal Crohn's disease refractory to biologic therapy. *Clin Gastroenterol Hepatol*. 2014;12(1):64–71.
35. Whiteford MH, Kilkenny J 3rd, Hyman N, Buie WD, Cohen J, Orsay C, Dunn G, Perry WB, Ellis CN, Rakinic J, et al. Practice parameters for the treatment of perianal abscess and fistula-in-ano (revised). *Dis Colon Rectum*. 2005; 48(7):1337–42.
36. Song YP, Fang BJ, Wei XD, Zheng S. [A preliminary study on mechanisms for resistance of CML patient BM-derived bcr/abl+ and Flk1+CD31-CD34- stem cells to ST1571 in vitro]. *Zhong Shi Yan Xue Ye Xue Za Zhi* 2005, 13(6):1004–1009.
37. 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(7):1416–23.
38. da Silva ML, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J Cell Sci*. 2006;119(Pt 11):2204–13.
39. 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(4):851–60.
40. Stang A, Jonas S, Poole C. Case study in major quotation errors: a critical commentary on the Newcastle-Ottawa scale. *Eur J Epidemiol*. 2018;33(11):1025–31.
41. Moskalewicz A, Oremus M. No clear choice between Newcastle-Ottawa scale and appraisal tool for cross-sectional studies to assess methodological quality in cross-sectional studies of health-related quality of life and breast cancer. *J Clin Epidemiol*. 2020;120:94–103.
42. Cumpston M, Li T, Page MJ, Chandler J, Welch VA, Higgins JP, Thomas J. Updated guidance for trusted systematic reviews: a new edition of the cochrane handbook for systematic reviews of interventions. *Cochrane Database Syst Rev*. 2019;10:Ed000142.
43. García-Olmo D, Herreros D, Pascual I, Pascual JA, Del-Valle E, Zorrilla J, De-La-Quintana P, García-Arranz M, Pascual M. Expanded adipose-derived stem cells for the treatment of complex perianal fistula: a phase II clinical trial. *Dis Colon Rectum*. 2009;52(1):79–86.
44. Guadalajara H, Herreros D, De-La-Quintana P, Trebol J, García-Arranz M, García-Olmo D. Long-term follow-up of patients undergoing adipose-derived adult stem cell administration to treat complex perianal fistulas. *Int J Color Dis*. 2012;27(5):595–600.
45. Herreros MD, García-Arranz M, Guadalajara H, De-La-Quintana P, García-Olmo D. Autologous expanded adipose-derived stem cells for the treatment of complex cryptoglandular perianal fistulas: a phase III randomized clinical trial (FATT 1: Fistula Advanced Therapy Trial 1) and long-term evaluation. *Dis Colon Rectum*. 2012;55(7):762–72.
46. García-Arranz M, García-Olmo D, Herreros MD, Gracia-Solana J, Guadalajara H, de la Portilla F, Baixauli J, García-García J, Ramirez JM, Sanchez-Guijo F, et al. Autologous adipose-derived stem cells for the treatment of complex cryptoglandular perianal fistula: a randomized clinical trial with long-term follow-up. *Stem Cells Transl Med*. 2020;9(3):295–301.
47. Molendijk I, Bonsing BA, Roelofs H, Peeters KC, Wasser MN, Dijkstra G, van der Woude CJ, Duijvestein M, Veenendaal RA, Zwaginga JJ, et al. Allogeneic

- bone marrow-derived mesenchymal stromal cells promote healing of refractory perianal fistulas in patients with Crohn's disease. *Gastroenterology*. 2015;149(4):918–927.e916.
48. Panés J, García-Olmo D, Van Assche G, Colombel JF, Reinisch W, Baumgart DC, Dignass A, Nachury M, Ferrante M, Kazemi-Shirazi L, 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. *Lancet*. 2016;388(10051):1281–90.
49. Panés J, García-Olmo D, Van Assche G, Colombel JF, Reinisch W, Baumgart DC, Dignass A, Nachury M, Ferrante M, Kazemi-Shirazi L, et al. Long-term efficacy and safety of stem cell therapy (cx601) for complex perianal fistulas in patients with Crohn's disease. *Gastroenterology*. 2018;154(5):1334–1342.e1334.
50. Zhou C, Li M, Zhang Y, Ni M, Wang Y, Xu D, Shi Y, Zhang B, Chen Y, Huang Y, et al. Autologous adipose-derived stem cells for the treatment of Crohn's fistula-in-ano: an open-label, controlled trial. *Stem Cell Res Ther*. 2020;11(1):124.
51. Barnhoorn MC, Wasser M, Roelofs H, Maljaars PWJ, Molendijk I, Bonsing BA, Oosten LEM, Dijkstra G, van der Woude CJ, Roelen DL, et al. Long-term evaluation of allogeneic bone marrow-derived mesenchymal stromal cell therapy for Crohn's disease perianal fistulas. *J Crohns Colitis*. 2020;14(1):64–70.
52. Avivar-Valderas A, Martín-Martín C, Ramírez C, Del Río B, Menta R, Mancheño-Corvo P, Ortiz-Virumbrales M, Herrero-Méndez Á, Panés J, García-Olmo D, et al. Dissecting allo-sensitization after local administration of human allogeneic adipose mesenchymal stem cells in perianal fistulas of Crohn's disease patients. *Front Immunol*. 2019;10:1244.
53. García-Olmo D, Guadalajara H, Rubio-Perez I, Herreros MD, de-la Quintana P, García-Arranz M. Recurrent anal fistulae: limited surgery supported by stem cells. *World J Gastroenterol*. 2015;21(11):3330–6.
54. Herreros MD, García-Olmo D, Guadalajara H, Georgiev-Hristov T, Brandariz L, García-Arranz M. Stem cell therapy: a compassionate use program in perianal fistula. *Stem Cells Int*. 2019;2019:6132340.
55. Choi S, Ryoo SB, Park KJ, Kim DS, Song KH, Kim KH, Chung SS, Shin EJ, Cho YB, Oh ST, et al. Autologous adipose tissue-derived stem cells for the treatment of complex perianal fistulas not associated with Crohn's disease: a phase II clinical trial for safety and efficacy. *Tech Coloproctol*. 2017;21(5):345–53.
56. Dozois EJ, Lightner AL, Mathis KL, Chua HK, Kelley SR, Fletcher JG, Dietz AB, Fritton JJ, Butler GW, Faubion WA. Early results of a phase I trial using an adipose-derived mesenchymal stem cell-coated fistula plug for the treatment of transsphincteric cryptoglandular fistulas. *Dis Colon Rectum*. 2019;62(5):615–22.
57. Topal U, Eray IC, Rencüzoğulları A, Yalav O, Alabaz Ö. Short-term results of adipose-derived stem cell therapy for the treatment of complex perianal fistula a single center experience. *Ann Ital Chir*. 2019;90:583–9.
58. Ciccocioppo R, Bernardo ME, Sgarella A, Maccario R, Avanzini MA, Ubezio C, Minelli A, Alvisi C, Vanoli A, Calliada F, et al. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. *Gut*. 2011;60(6):788–98.
59. 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(2):279–85.
60. 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 multicenter phase I/IIa clinical trial. *Int J Color Dis*. 2013;28(3):313–23.
61. Lee WY, Park KJ, Cho YB, Yoon SN, Song KH, Kim DS, Jung SH, Kim M, Yoo HW, Kim I, et al. Autologous adipose tissue-derived stem cells treatment demonstrated favorable and sustainable therapeutic effect for Crohn's fistula. *Stem Cells*. 2013;31(11):2575–81.
62. Ciccocioppo R, Gallia A, Sgarella A, Kruzliak P, Gobbi PG, Corazza GR. Long-term follow-up of Crohn disease fistulas after local injections of bone marrow-derived mesenchymal stem cells. *Mayo Clin Proc*. 2015;90(6):747–55.
63. Cho YB, Park KJ, Yoon SN, Song KH, Kim DS, Jung SH, Kim M, Jeong HY, Yu CS. Long-term results of adipose-derived stem cell therapy for the treatment of Crohn's fistula. *Stem Cells Transl Med*. 2015;4(5):532–7.
64. Dietz AB, Dozois EJ, Fletcher JG, Butler GW, Radel D, Lightner AL, Dave M, Fritton J, Nair A, Camilleri ET, 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(1):59–62.e52.
65. Lightner AL, Dozois EJ, Dietz AB, Fletcher JG, Fritton J, Butler G, Faubion WA. Matrix-delivered autologous mesenchymal stem cell therapy for refractory rectovaginal Crohn's fistulas. *Inflamm Bowel Dis*. 2020;26(5):670–7.
66. Dige A, Hougaard HT, Agnholt J, Pedersen BG, Tencerova M, Kassem M, Krogh K, Lundby L. Efficacy of injection of freshly collected autologous adipose tissue into perianal fistulas in patients with Crohn's disease. *Gastroenterology*. 2019;156(8):2208–2216.e2201.
67. Park KJ, Ryoo SB, Kim JS, Kim TI, Baik SH, Kim HJ, Lee KY, Kim M, Kim WH. Allogeneic adipose-derived stem cells for the treatment of perianal fistula in Crohn's disease: a pilot clinical trial. *Colorectal Dis*. 2016;18(5):468–76.
68. García-Arranz M, Herreros MD, González-Gómez C, de la Quintana P, Guadalajara H, Georgiev-Hristov T, Trébol J, García-Olmo D. Treatment of Crohn's-related rectovaginal fistula with allogeneic expanded-adipose derived stem cells: a phase I-IIa clinical trial. *Stem Cells Transl Med*. 2016; 5(11):1441–6.
69. Scott LJ. Darvadstrocel: a review in treatment-refractory complex perianal fistulas in Crohn's disease. *BioDrugs*. 2018;32(6):627–34.
70. Wainstein C, Quera R, Fluxá D, Kronberg U, Conejero A, López-Köstner F, Jofre C, Zarate AJ. Stem cell therapy in refractory perineal Crohn's disease: long-term follow-up. *Colorectal Dis*. 2018.
71. Duijvestein M, Vos AC, Roelofs H, Wildenberg ME, Wendrich BB, Verspaget HW, Kooy-Winkelaar EM, Koning F, Zwaginga JJ, Fidder HH, et al. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. *Gut*. 2010; 59(12):1662–9.
72. Tan J, Wu W, Xu X, Liao L, Zheng F, Messinger S, Sun X, Chen J, Yang S, Cai J, et al. Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized controlled trial. *Jama*. 2012; 307(11):1169–77.
73. Reinders ME, Dreyer GJ, Bank JR, Roelofs H, Heidt S, Roelen DL, Zandvliet ML, Huurman VA, Fibbe WE, van Kooten C, et al. Safety of allogeneic bone marrow derived mesenchymal stromal cell therapy in renal transplant recipients: the neptune study. *J Transl Med*. 2015;13:344.
74. Muraro PA, Douek DC, Packer A, Chung K, Guenaga FJ, Cassiani-Ingoni R, Campbell C, Memon S, Nagle JW, Hakim FT, et al. Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients. *J Exp Med*. 2005;201(5):805–16.
75. Shi Y, Wang Y, Li Q, Liu K, Hou J, Shao C, Wang Y. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. *Nat Rev Nephrol*. 2018;14(8):493–507.
76. Clevers H, Loh KM, Nusse R. Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. *Science*. 2014;346(6205):1248012.
77. Discher DE, Mooney DJ, Zandstra PW. Growth factors, matrices, and forces combine and control stem cells. *Science*. 2009;324(5935):1673–7.
78. Panés J, Rimola J. Perianal fistulizing Crohn's disease: pathogenesis, diagnosis and therapy. *Nat Rev Gastroenterol Hepatol*. 2017;14(11):652–64.
79. Schwartz DA, Ghazi LJ, Regueiro M, Fichera A, Zoccali M, Ong EM, Mortelé KJ. Guidelines for the multidisciplinary management of Crohn's perianal fistulas: summary statement. *Inflamm Bowel Dis*. 2015;21(4):723–30.
80. American Gastroenterological Association medical position statement: perianal Crohn's disease. *Gastroenterology* 2003, 125(5):1503–1507.
81. García-Olmo D, García-Arranz M, Herreros D. Expanded adipose-derived stem cells for the treatment of complex perianal fistula including Crohn's disease. *Expert Opin Biol Ther*. 2008;8(9):1417–23.
82. Mizuno H, Zuk PA, Zhu M, Lorenz HP, Benhaim P, Hedrick MH. Myogenic differentiation by human processed lipoaspirate cells. *Plast Reconstr Surg*. 2002;109(1):199–209 discussion 210–191.
83. Vieira NM, Brandalise V, Zucconi E, Secco M, Strauss BE, Zatz M. Isolation, characterization, and differentiation potential of canine adipose-derived stem cells. *Cell Transplant*. 2010;19(3):279–89.
84. Khanna R, Zou G, D'Haens G, Feagan BG, Sandborn WJ, Vandervoort MK, Roller RL, Bortey E, Paterson C, Forbes WP, et al. A retrospective analysis: the development of patient reported outcome measures for the assessment of Crohn's disease activity. *Aliment Pharmacol Ther*. 2015;41(1):77–86.
85. Ryska O, Serclouva Z, Mestak O, Matouskova E, Vesely P, Mrázova I. Local application of adipose-derived mesenchymal stem cells supports the healing of fistula: prospective randomised study on rat model of fistulising Crohn's disease. *Scand J Gastroenterol*. 2017;52(5):543–50.
86. Meng ZW, Baumgart DC. Darvadstrocel for the treatment of perianal fistulas in Crohn's disease. *Expert Rev Gastroenterol Hepatol*. 2020;14(6):405–10.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.