Published in final edited form as:

Inflamm Bowel Dis. 2015 November ; 21(11): 2696–2707. doi:10.1097/MIB.0000000000000543.

Mesenchymal Stem Cell Therapy for Inflammatory Bowel Disease: A Systematic Review and Meta-analysis

Maneesh Dave, M.B.B.S., M.P.H.¹, Kathan Mehta, M.B.B.S., M.P.H.², Jay Luther, MD³, Anushka Baruah, M.B.B.S.⁴, Allan B Dietz, PhD⁵, and William A. Faubion Jr., M.D.⁶

¹Division of Gastroenterology & Liver Disease, Case Western Reserve University, University Hospitals Case Medical Center, Cleveland, Ohio, USA

²Division of Internal Medicine, University of Pittsburgh Medical Centre Shadyside Hospital, Pennsylvania

³Division of Gastroenterology & Hepatology, Massachusetts General Hospital, Boston, Massachusetts, USA

⁴Division of Internal Medicine, John Stroger Hospital of Cook County, Chicago, Illinois, USA

⁵Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA

⁶Division of Gastroenterology & Hepatology, Mayo Clinic, Rochester, Minnesota, USA

Abstract

Recent advances in inflammatory bowel disease (IBD) therapeutics include novel medical, surgical, and endoscopic treatments. Among these, stem cell therapy is still in its infancy, though multiple studies suggest the immunomodulatory effect of stem cell therapy may reduce inflammation and tissue injury in IBD patients. This review discusses the novel avenue of stem cell therapy and its potential role in the management of ulcerative colitis and Crohn's disease. We conducted a comprehensive literature search to identify studies examining the role of stem cell therapy (without conditioning and immunomodulatory regimens) in IBD. Taken together these studies suggest a promising role for SCT in IBD, although the substantial challenges, such as cost and inadequate/incomplete characterization of effect limit their current use in clinical practice.

Keywords

Inflammatory bowel disease; Mesenchymal stem cells; Systematic review and meta-analysis; adverse effects; efficacy

Correspondence: Maneesh Dave, Division of Gastroenterology & Liver Disease, University Hospitals Case Medical Center, 11100 Euclid Avenue, Cleveland, OH-44106, USA. Tel: 216-844-1995; Fax: 216-844-7371; maneesh.dave@uhhospitals.org, William A Faubion, M.D., Division of Gastroenterology and Hepatology, Guggenheim 10, 200 1st St. SW, Rochester, MN 55905, USA. Tel: 507-284-2468; Fax: 507-255-6318; faubion.william@mayo.edu.

Disclosures: The authors have nothing to disclose.

Writing assistance: Not applicable.

Author contributions: Study concepts/study design: MD and WAF, literature research, M.D., K.M., J.L., A.B.; data acquisition or data analysis/interpretation, MD, K.M., J.L.; statistical analysis, MD and K.M.; manuscript drafting: MD and K.M.; manuscript revision for important intellectual content, MD, WAF, K.M., A.D. and J.L.; manuscript final version approval, all authors.

INTRODUCTION

Inflammatory bowel disease (IBD) which comprises of Ulcerative Colitis (UC) and Crohn's Disease (CD), is presumed to result from an inappropriate response of the host's immune system to intestinal microbes ¹. The existing management strategies for IBD therefore target inflammation and include immunosuppressive therapy with corticosteroids, azathioprine, 6 mercaptopurine (6-MP), monoclonal antibodies against cytokine tumour necrosis factor alpha (infliximab, adalimumab, certolizumab pegol and golimumab), the recently approved integrin inhibitor Vedolizumab (anti- $\alpha 4\beta 7$ Integrin), and surgery ²⁻¹³. A recent study of monotherapy versus combination therapy in UC patients showed induction of clinical remission (defined as a total Mayo score of 2 points or less, with no individual subscore exceeding 1 point, without the use of corticosteroids) in 23.7% by azathioprine, in 22.1% by infliximab and in only 39.7% of patients by combination therapy at 16 weeks ¹⁴. Thus, approximately 60% of UC patients failed to achieve clinical remission at 16 weeks even with combination therapy. Similarly, the SONIC study (Study of Biologic and Immunomodulator Naive Patients in Crohn's Disease) for CD patients, showed induction of clinical remission in 30% of patients by azathioprine, in 44.4% by infliximab and in 56.8% of patients by combination therapy at 26 weeks. Therefore, combination therapy fails to achieve clinical remission in 43% of patients at the end of 26 weeks ¹⁵. In addition, use of these immunosuppressive medications is associated with risk of adverse events like serious infections ¹⁶, neurological disorders ¹⁷ and malignancies ¹⁸. Thus, there is a need for novel and new therapies to treat IBD.

Over the last three decades, tremendous progress has been made in the field of regenerative medicine and stem cell biology ¹⁹. Stem cells are a unique group of undifferentiated cells that have capacity of self-renewal, and under certain physiological or experimental conditions possess the ability to become organ or tissue specific cells with differentiated function ²⁰. In addition to their regenerative properties, a type of stem cells called multipotent mesenchymal stem cells (MSC) have unique immunoregulatory/suppressive properties that are currently being investigated as a treatment option for inflammatory disorders. In this study, we perform a systematic review of adverse events and efficacy of MSC's for treatment of IBD. In order to provide relevant context, we briefly describe different types of stem cells, and their putative *in-vitro* and *in-vivo* mechanisms of immunoregulation.

Stem cells can be broadly categorized as embryonic or adult-derived stem cells. The embryonic stem (ES) cells are undifferentiated primitive cells derived from the preimplantation embryo with capability of dividing without differentiating or developing changes in karyotypes for a prolonged period of time. These ES cells may differentiate into specialized tissue *in-vivo* and *in-vitro* ²¹. ES cells are known to develop into cells and tissues of the three primary germ layers that ultimately lead to formation of an organism. As the development proceeds, the ES cells disappear by differentiating into specialized tissue, however a small number of stem cells are retained in the specialized tissues which are known as adult stem cells or somatic stem cells ²². The adult stem cells, in contrast to ES cells possess a limited capacity for self-renewal and differentiation, a process usually limited to the organ of origin. Among adult stem cells, the best-defined cells are the hematopoietic

stem cells (HSC), mesenchymal stem cells (MSC) and intestinal stem cells (ISC). HSCs are stem cells isolated from the blood and bone marrow and possess the ability to give rise to red blood cells, white blood cells and platelets²³. HSCs are under study in the treatment of IBD^{24, 25}. The preliminary results of the ASTIC trial (Autologous Stem Cell Transplantation International Crohn's Disease) suggests that immunoablation and hematopoietic stem cell transplantation (HSCT) may be effective in treating CD; but high rates of serious adverse events may make it unsuitable for the vast majority of patients²⁶. The ISCs are located at the base of crypt of Lieberkühn and are positive for Leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5+)²⁷. The ISCs play key role in regeneration of intestinal epithelium and are focus of intense investigations²⁷.

MSCs are non-hematopoietic multi-potent stem cells that have the capacity to differentiate into a limited array of differentiated cell types of mesodermal lineage including chondrocytes, osteoblasts, and adipocytes under different micro-environmental conditions, culture media and supplements²⁸. MSCs have been isolated from various tissues including the bone marrow, adipose tissue and human umbilical cord²⁹. Their properties include *in-vitro* proliferation with plastic-adherent properties bearing fibroblast-like morphology, the expression of mesenchymal stem cell markers and stem cell specific genes, the ability to form colonies and differentiate into various cell lineages^{30–33}. Recently, MSCs have been demonstrated to have immunoregulatory properties by suppression of allogeneic lymphocyte proliferation when added to mixed lymphocyte reaction and in mouse models of inflammation^{34, 35}. The immunomodulation by MSCs is thought to be a multi stage process³⁶. MSCs have been shown to migrate to sites of inflammation in response to stromal cell derived factor (SDF) –1 alpha³⁷ or secondary lymphoid tissue chemokines *in-vivo*³⁸ and complement proteins, growth factors, and chemokines *in-vitro*^{39, 40}. At the site of inflammation, the MSCs have been shown to require activation by interferon-gamma (IFN- γ), tumor necrosis factor alpha (TNF α) or interleukin 1 beta (IL-1 β) in order to exert immunosuppressive effect^{41, 42}. The activated MSCs produce immunosuppressive soluble factors including indoleamine 2,3- dioxygenase (IDO), prostaglandin E2 (PGE2), TNF - alpha stimulated protein/ gene 6 (TSG-6), and nitric oxide (NO) leading to macrophage polarization to the anti-inflammatory M2 phenotype, induction of tolerogenic dendritic cells (DCs) and T regulatory cells *in-vivo*, promotion of Th1–Th2 switch, inhibition of mast cell and Th17 differentiation as well as induction of T-cell apoptosis^{41, 43–45}. MSCs have also been shown to interfere with dendritic cell (DC) function (migration, maturation and antigen presentation) by variety of mechanisms including down regulation of DC maturation markers like major histocompatibility complex (MHC) class II, CD40, CD80 and CD86^{46–50}. MSCs also exert their immunomodulation by a number of contact dependent mechanisms involving FAS/FAS ligand, programmed death-1/programmed death ligand-1, galectins, CD39-induced adenosine and Notch signaling^{49–53}(Figure 1).

Given the *in-vivo* and *in-vitro* immunoregulatory potential of MSC, multiple studies have been conducted to assess safety and efficacy of stem cell (MSC) therapy in IBD. Two forms of MSC therapy have been utilized, one involves systemic infusion of stem cells for CD and UC, and the other involves localized application of stem cells for perianal CD. In this study we perform a systematic review and meta-analysis of safety and efficacy of stem cell

therapy without the use of any conditioning, myeloablation or total body radiation for treatment of IBD.

METHODS

We followed the standard Cochrane guidelines and the PRISMA statement for performing and reporting systematic review ^{54, 55}.

Search strategy

A systematic review of English and non- English articles was performed using PubMed (since inception to March 2015) and EMBASE (since inception to November 2014). The search was performed independently by the authors (MD, KM and JL), and by an information library specialist (Larry Prokop). We also identified additional studies by searching bibliographies and abstracts presented at the Digestive Disease Week, American College of Gastroenterology and United European Gastroenterology Week from 2005 to 2014. We used free text words and MeSH terms with and without Boolean operators (“AND”, “OR”) to increase the sensitivity of the search ⁵⁶. The detailed search strategy is available in supplementary table 1.

Study selection

Studies were selected based on the following inclusion criteria: (i) Human studies (ii) Included patients with IBD (iii) MSCs were used for treatment of IBD (iii) No preparatory regimen for immunosuppression that is whole body irradiation or myeloablation (iv) Efficacy and adverse events were reported (v) The study was published as peer reviewed paper, letter or abstract. Exclusion criteria were i) Non human studies ii) Use of total body irradiation or myeloablative regimen.

Data extraction

Two independent reviewers (K.M & M.D) extracted data from the selected studies using standardized data extraction forms. These forms included: a) Author b) Journal c) Year of publication d) Country where study was performed e) Type of study f) Sample size g) Number of CD cases and UC cases h) Number of healthy controls (if any) i) Type and source of stem cells j) Primary outcome k) Efficacy outcome and m) Adverse events.

Statistical analysis

The primary outcomes of this analysis were proportion of patients with healed fistula after local injection of MSCs as defined by the study investigators and proportion of patients with induction of remission after systemic infusion of MSCs. Freeman-Tukey transformation ⁵⁷ was used to calculate pooled proportions under the fixed and random effects model ⁵⁸. The heterogeneity (i.e, between-study variation) of the results between the studies was assessed graphically by using chi square test of homogeneity and the inconsistency index (I^2) ⁵⁹. Inconsistency indexes describe the percentage of total variation across studies that are due to heterogeneity rather than to chance. An inconsistency index of 0% indicates no observed heterogeneity, <40% as low heterogeneity and values greater than 50% may be considered to indicate substantial heterogeneity ⁶⁰. Sensitivity analysis was performed by excluding one

study at a time to reflect influence of individual study on pooled proportion. Statistical analysis was performed in R statistical software⁶¹ using “meta” package for meta-analysis⁶².

Assessment of study quality and bias

The methodological quality of the studies was assessed by two investigators (M.D. and K.M.), independently using Cochrane risk of bias tool⁵⁵. The studies were scored across five categories: selection bias, performance bias, detection bias, attrition bias, and reporting bias.

Results

Search results

The initial search strategy yielded 4828 abstracts for review, of which 36 were selected for detailed review and 12 met the inclusion criteria (Supplementary figure 1). Twenty-three studies were excluded for being non-human studies or using myeloablative preparatory regimen or radiation. A study by Herreros et al.⁶³ was excluded; as it was a randomized, single blind trial of ASCs vs. fibrin glue vs. ASCs plus fibrin glue in 200 patients with cryptoglandular perianal fistulas and did not include IBD patients. We first report the efficacy and safety of stem cells for perianal CD which involved local application of stem cells and then report the efficacy and safety of stem cells for luminal IBD separately as that involved systemic infusion of stem cells.

Efficacy of SCT for Peri-anal CD (Local therapy)

Garcia-Olmo et al., in 2003, were the first investigators to report a case of successful healing of perianal fistula in a CD patient (who previously failed multiple immunosuppressive therapies) by local injection of autologous adipose derived stem cells (ASCs)⁶⁴. The same group later conducted a phase I trial of local injections of autologous ASCs in five CD patients who had rectovaginal, perianal and/or enterocutaneous fistulas. The study demonstrated complete closure in three out of four rectovaginal or perianal fistula (75%) and three out of four enterocutaneous fistulas (75%) at eight weeks post treatment⁶⁵.

Fibrin glue alone has often been tried to close peri-anal fistulas, however, previous meta-analyses and systematic reviews had showed no significant difference between outcomes of fibrin glue vs. conventional surgery^{66,67}. The study by Garcia-Olmo et al. showed that a combination of autologous ASCs and fibrin glue produced better results than fibrin glue alone⁶⁸. This randomized, open label trial studied seven patients with CD who received fibrin glue alone compared to 7 CD patients who received fibrin glue plus 2 million ASCs. If the fistula had not healed at 8 weeks, the patients received a second dose of fibrin glue or fibrin glue plus 4 million ASCs in respective arms. Five out of seven patients (71%) in stem cells plus fibrin glue group had healed fistulas (defined as closure of external opening and absence of suppuration upon pressure) compared to one out of seven patients (14%) in fibrin glue alone group at 8 weeks after last treatment. While the study showed effectiveness of ASC plus fibrin glue over fibrin glue alone (relative risk = 5), the results were not statistically significant for CD patients ($p=0.1$). Ciccocioppo R. et al performed a study to

determine fistula closure by local injection of bone marrow derived autologous MSCs in 10 patients with CD ⁶⁹. One patient had multiple enterocutaneous fistulas and others had complex perianal fistula. The patients were treated with serial local injection of MSCs every 4 weeks over a 16-week period, on an average of 4 doses (range 2–5). Six out of 9 patients with complex perianal fistula (67%) and one patient with multiple enterocutaneous fistulas had complete closure evident on clinical exam as well as MRI. A study by de la Portilla et al. used expanded allogeneic ASCs (eASCs) and demonstrated the effectiveness of local injections of eASCs in CD patients with peri-anal fistulas ⁷⁰. Twenty-four patients received 20 million eASCs in draining fistula tract by local injection. If fistula closure was incomplete at week 12, a second dose of 40 million eASCs was given. The fistula closure was strictly defined as: absence of suppuration through the external orifice and complete re-epithelization, plus absence of collections measured by MRI. Five out of 18 fistulas closed (28%) on per-protocol analysis; and 7 out of 18 patients (47%) had closure of external openings at 24 weeks post treatment. A phase I clinical study conducted by Cho et al used autologous ASCs for treatment of fistulizing CD and defined fistula healing as complete closure of the fistula track, and internal and external openings without drainage or signs of inflammation ⁷¹. Three out of 10 patients had complete healing of fistula at 8 weeks post treatment. This was followed by phase II study from the same group, which is the largest MSC study for perianal CD to date and confirmed the effectiveness of autologous ASCs in fistulizing CD ⁷². Twenty seven out of 33 patients (82%) had complete fistula healing at 8 weeks post treatment defined as complete closure of the fistula track, and internal and external openings without drainage or signs of inflammation. The authors recently published a follow up study in which the patients were followed for 2 years regardless of response in the initial trial⁷³. The study showed that at 24 months, by modified per-protocol analysis 21 out of 26 patients (80.8%) had sustained closure of the fistula.

A meta-analysis of all perianal CD studies showed that 61.3% (95% CI 35.6% – 84.6%) of patients had healed fistula after local MSC administration (Figure 3). However, the included studies had high heterogeneity ($I^2 = 68.9\%$). Sensitivity analysis performed by excluding studies sequentially showed that by excluding the de la Portilla et al. ⁷⁰ study the heterogeneity decreased to moderate level ($I^2 = 44.3\%$) and by excluding the Lee et al.⁷² study the heterogeneity decreased to moderate level ($I^2 = 41.5\%$). While, de la Portilla et al. used MRI to define fistula closure, they also reported number of patients who had closure of external opening of fistula. When we used number of patients with closed external opening (7 of 18) for the meta-analysis, the heterogeneity (I^2) reduced to 58.5%. MRI is considered as standard method of fistula healing in clinical trials by ECCO ⁷⁴ as it shows residual inflammation in the fistula track despite the external closure of the fistula; only two out of seven studies have used MRI to evaluate healing of the fistula. Table 1 gives more details of the studies and efficacy outcomes.

Safety of SCT for Peri-anal CD (Local therapy)

The case report, phase I and Phase II studies by Garcia-Olmo et al reported no serious adverse events (AEs) related to autologous ASCs administration ^{64, 65, 68}. The duration of follow up was 3 months, 22 months and 12 months respectively. Ciccocioppo R. et al again reported no AEs in 12 months of follow up after administration of bone marrow derived

autologous MSCs⁶⁹. However, the study by Portilla et al. which followed the patients for median 211 days (IQR 186–267 days) reported two serious AEs (fever and peri-anal abscess) after administration of allogeneic ASCs which lead to withdrawal of two patients (8%) from the study⁷⁰. The phase I study of autologous ASCs by Cho et al. which followed the patients for 8 months also reported three serious AEs (enterocolitis, seton application and infliximab administration for new fistulas unrelated to target fistula) in two patients (20%) requiring hospitalization⁷¹. Notably, the Phase II study of autologous ASCs by Lee et al. reported no serious AEs in 12 months of follow-up period⁷². Thus, two out of seven studies reported serious AEs after MSC administration, and both SAE were potentially related to underlying CD than treatment with MSC.

Minor adverse reaction reported in phase II study of autologous ASCs by Garcia-Olmo et al. included peri-anal sepsis (12 percent) in 12 months of follow-up period⁶⁸. The phase I study of autologous ASCs by Cho et al. which followed the patients for 8 months reported two non-serious AEs (pain at the site of injection and diarrhea) in five patients (50%)⁷¹. The phase II study of autologous ASCs by Lee et al. followed the thirty-three CD patients for 12 months and reported postoperative pain, anal pain, and anal bleeding in 60%, 19%, and 7% of patients respectively after administration of autologous ASCs, none of which led to hospitalization. One patient was hospitalized for vitamin B12 deficiency. Two patients were hospitalized for exacerbation of CD and peritonitis respectively, which were deemed not related to ASC administration⁷². Overall, the studies have demonstrated that administration of MSCs can lead to minor adverse reaction like perianal sepsis; but serious adverse reactions leading to hospitalization are less common and perhaps related to underlying CD activity.

Efficacy of SCT as systemic infusion for IBD

Onken et al. administered allogeneic bone marrow derived MSCs by systemic infusion in 10 patients with active luminal CD refractory to steroids and immunomodulators (CRP $\geq$ 5 mg/L, CDAI $\geq$ 220) without performing any donor-recipient matching⁷⁵. Five patients were randomly assigned to receive high dose MSCs (8 million cells/kg), and 5 received low dose MSCs (2 million cells/kg). The study demonstrated significant decline in Crohn's Disease Activity Index (CDAI) by greater than 100 points in 3 patients at 14 days post treatment. One patient also achieved clinical remission (CDAI $<$ 150) by day 7. The mean IBDQ score also increased significantly by Day 28 (113 vs. 146, $p=0.008$). Duijvestein et al. administered autologous bone-marrow derived MSCs in 2 doses of 1–2 million cells/kg, one week apart, in 10 patients with luminal CD refractory to steroids and immunomodulators. The study demonstrated a drop in CDAI by $\geq$ 70 from baseline in 3 patients at 6 weeks post treatment⁷⁶. However, none achieved remission. Lazebnik et al. also used allogeneic bone marrow derived MSCs and cultured cells for 5–6 weeks to get donor MSC in a quantity of $(1.5-2) \times 10^8$. The study demonstrated statistically significant decline in CDAI, Rahmilevich Clinical Activity Index (RCAI), Mayo and Gebs scales following intravenous administration of MSCs in 31 out of 39 patients with UC and 9 out of 11 patients with CD after 4 to 8 months of follow up⁷⁷. While all 50 patients were on steroids before the administration of MSCs, 34 of them were able to taper off steroids after MSCs administration. Liang et al. administered allogeneic bone marrow derived MSCs (3 cases)

and umbilical cord derived MSCs (4 cases), in 4 patients with CD and 3 patients with UC. The patients continued steroids and/or immunomodulators after the MSC infusion. At the 3-month visit, five patients achieved remission and maintenance of remission lasted for more than 24 months in two patients ⁷⁸. However, the methods of culture of MSCs and definitions of remission were unclear. Forbes et al. also used allogeneic bone marrow derived MSCs and gave weekly infusions of 2 million cells/kg body weight for 4 weeks to patient with active luminal CD refractory to immunomodulators. The study demonstrated significant decline in CDAI by > 100 in 12 out of 15 patients (80%, 95% confidence interval, 72%–88%), and induction of remission (defined as CDAI < 150) in 8 patients (53%, 95% confidence interval, 43%–64%), at 42 days after intravenous administration of MSCs ⁷⁹.

A meta-analysis of all studies of SCT as systemic infusion showed that 40.5% (95% CI 7.5% – 78.5%) of patients achieved remission after infusion of MSCs (Figure 4). The studies included had high heterogeneity ($I^2 = 90.3\%$) and a sensitivity analysis performed by excluding studies sequentially did not decrease the heterogeneity. Subgroup analysis by disease i.e CD or UC also did not decrease the high heterogeneity.

Safety of SCT as systemic infusion for IBD

The study of allogeneic bone marrow derived MSCs by Onken et al. followed ten CD patients for 28 days and reported one unrelated serious AE (anemia, 10%). There were no infusion reactions related to MSCs administration ⁷⁵. Another study of allogeneic bone marrow derived MSCs by Forbes et al. followed fifteen CD patients for 6 weeks and reported one serious AE (adenocarcinoma arising in a dysplasia associated lesion or mass) in one patient (6%). By retrospective charts reviews, the authors suspected that the cancer was present even before the MSC administration and the patient should not have been allowed to enter the study. However, the authors concluded that possibility of MSC contributing to progression of dysplasia to cancer could not be entirely excluded ⁷⁹. The study of autologous bone-marrow derived MSCs by Duijvestein et al. also reported no serious AEs in 6 months of follow up period ⁷⁶. The study of allogeneic bone marrow derived MSCs and umbilical cord derived MSCs by Liang et al. reported no serious AEs in 19 months of mean follow-up period ⁷⁸. Thus, serious AEs related to MSC are relatively uncommon.

The study of allogeneic bone marrow derived MSCs by Onken et al. which followed ten CD patients for 28 days reported five patients (50%) with non-serious AEs after systemic infusion of MSCs. The exact details of minor adverse reactions were not available. The study of autologous bone-marrow derived MSCs by Duijvestein et al. followed ten CD patients for 6 months and reported minor allergic reaction (10%), headache (30%), and taste and smell disturbances (90%) which were considered related to MSCs infusion. They also reported worsening of CD requiring hospitalization (20%), abdominal pain (30%), nausea (20%), fatigue (20%), anorexia (20%), dizziness (10%), vomiting (10%), bloating (10%), fever (10%), diarrhea (10%), common cold (10%), otitis media (10%); all of which were considered unrelated to MSCs infusion ⁷⁶. In the study of allogeneic bone marrow derived MSCs by Lazebnik et al., 12 patients (24%) had mild transfusion reaction following the administration of MSCs which included fever, arthralgia, and myalgia ⁷⁷. Two patients (4%) developed hives and angioedema that were managed by using antihistamines and

corticosteroids. The study of allogeneic bone marrow derived MSCs and umbilical cord derived MSCs by Liang et al. followed seven patients for mean of 19 months (range 6–32) and reported three non-serious AEs (feeling of hot in face, insomnia, and increase in frequency of diarrhea associated with low grade fever) which did not require hospitalization ⁷⁸. The study of allogeneic bone marrow derived MSCs by Forbes et al. followed fifteen CD patients for 6 weeks and reported dysgeusia (100%), headache (19%), lymphopenia (19%), increased alanine aminotransferase (19%), self-limiting infection (13%), and nausea (1%) which did not require hospitalization ⁷⁹. Thus, commonly reported non-serious AEs after systemic infusion of MSCs were headache, diarrhea, mild transfusion reaction and taste and smell disturbances. All of the non-serious AEs were self limited.

Assessment of Study Quality

There was overall high risk of performance bias, detection bias, attrition bias and reporting bias in the studies included in the review (Figure 2A and 2B).

Discussion

Our systematic review and meta-analysis suggests that SCT has good therapeutic potential with low risk of adverse events for IBD patients, particularly for those who have perianal disease and are treated with local therapy. The major advantage of mesenchymal SCT is that it does not require preparatory regimens involving high dose chemotherapy and/or radiation like bone marrow/hematopoietic stem cell transplant. As a result, it is associated with less adverse events and procedure related mortality. MSCs have regenerative and immunomodulatory properties which lead to reduction of inflammation and healing of affected intestinal tissue. Due to immunomodulatory properties of MSCs, healthy donors (instead of IBD patients) can also be used as a source of MSCs without increasing risk of rejection by the host.

Several limitations temper the findings of our analysis. First, out of 12 studies included in this review, only one study was randomized double arm trial comparing fibrin glue alone vs fibrin glue plus SCT. Second, studies used different sources of stem cells including adipose tissue, bone marrow and umbilical cord from same patient (autologous) as well as healthy volunteers (allogeneic). Third the efficacy of local injection of SCT in closure of fistula varied widely between 28% to 82%, among the studies. Fourth, the method of assessment of fistula closure and follow up period varied among the studies. Only two studies used MRI for assessment of fistula closure and the rest relied on clinical exam. Last, adverse events reporting was not standardized. Four out of 7 studies reported minor local reactions like perianal sepsis but serious adverse events requiring hospitalization were rare. There was one reported case of malignancy, an established complication of IBD. The commonly observed minor adverse events were dysgeusia, headache and diarrhea; but serious adverse events requiring hospitalization were rare. However, the definitions of adverse events used among studies varied significantly. Moreover, the SCT related and SCT unrelated AEs were not defined precisely.

A recent phase III study utilizing MSC's to treat graft-versus-host disease (GVHD) failed to meet its primary end point ⁸⁰. In the failure analysis of the GVHD study, Galipeau argued

that immunoregulatory properties of MSCs vary between different donors (donor variance). The excessive expansion and replication of MSCs could also lead to lesser efficacy. Some recipients might have pre-existing alloimmunization leading to rejection of administered MSCs. Moreover, practice of cryopreservation till the day of procedure and thawing of MSCs several hours prior to administration could also lead to reduced efficacy⁸¹. All these arguments can also be applied to MSC therapy for IBD. In addition, several novel immunomodulatory properties of MSCs have been recently identified which have not yet been implemented in clinical studies. Pretreatment of MSCs with IFN- γ has been shown to enhance therapeutic effects of MSCs in mouse model of colitis⁸². Similarly, pre-exposure of umbilical cord blood MSCs with muramyl dipeptide (MDP) has been shown to increase ability of MSCs to suppress mononuclear cell proliferation, and in turn reduce the inflammation⁸³. MSCs with budesonide loaded Poly lactic-co-glycolic acid (PLGA) microparticles have been shown to exhibit enhanced IDO activity leading to increased suppression of peripheral blood mononuclear cells⁸⁴. Furthermore, adult stem cells other than MSC have recently been shown to exert an immunosuppressive effect⁸⁵. Thus these advances in preclinical studies need to be evaluated in treatment of IBD patients to increase the efficacy of MSC therapy.

Clinical trials that are currently underway (Supplementary table 2) to ascertain the safety and therapeutic benefits of mesenchymal stem cell therapy in IBD patients appear promising. However, they need to use standardized definitions of the side effects resulting directly from MSC therapy and incorporate more objective definitions of IBD remission like endoscopy, C reactive protein, fecal calprotectin and imaging including MRI for perianal CD.

Conclusion

MSCs are emerging as an alternative treatment for refractory IBD. Although, MSCs appear safe and potentially effective in initial studies, more studies in preclinical animal models and human studies that incorporate randomized controlled design are needed. Recent basic science advances in biology of MSCs needs to be incorporated in clinical trials to improve the efficacy of MSCs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

Grant Support: WAF's laboratory is supported by National Institutes of Health grants R01 AI089714. The funding agencies had no role in the study analysis or writing of the manuscript. Its contents are solely the responsibility of the authors.

We thank Natalia Aladyshkina (Case Western Reserve University) for translating the text in some studies to English.

References

1. Dave M, Papadakis KA, Faubion WA Jr. Immunology of Inflammatory Bowel Disease and Molecular Targets for Biologics. *Gastroenterol Clin North Am.* 2014; 43:405–424. [PubMed: 25110250]
2. Sandborn WJ, Feagan BG, Rutgeerts P, et al. Vedolizumab as induction and maintenance therapy for Crohn's disease. *N Engl J Med.* 2013; 369:711–721. [PubMed: 23964933]
3. Feagan BG, Rutgeerts P, Sands BE, et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* 2013; 369:699–710. [PubMed: 23964932]
4. Poggioli G, Pierangeli F, Laureti S, et al. Review article: indication and type of surgery in Crohn's disease. *Aliment Pharmacol Ther.* 2002; 16(Suppl 4):59–64. [PubMed: 12047262]
5. Nicholls RJ. Review article: ulcerative colitis--surgical indications and treatment. *Aliment Pharmacol Ther.* 2002; 16(Suppl 4):25–28. [PubMed: 12047256]
6. van Dulleman HM, van Deventer SJ, Hommes DW, et al. Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). *Gastroenterology.* 1995; 109:129–135. [PubMed: 7797011]
7. Hanauer SB, Feagan BG, Lichtenstein GR, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet.* 2002; 359:1541–1549. [PubMed: 12047962]
8. Jilani NZ, Akobeng AK. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. Colombel JF, Sandborn WJ, Rutgeerts P et al. *Gastroenterology* 2007;132:52–65. *J Pediatr Gastroenterol Nutr.* 2008; 46:226–227. [PubMed: 18223389]
9. Sandborn WJ, Feagan BG, Stoinov S, et al. Certolizumab pegol for the treatment of Crohn's disease. *N Engl J Med.* 2007; 357:228–238. [PubMed: 17634458]
10. O'Donoghue DP, Dawson AM, Powell-Tuck J, et al. Double-blind withdrawal trial of azathioprine as maintenance treatment for Crohn's disease. *Lancet.* 1978; 2:955–957. [PubMed: 81986]
11. Present DH, Korelitz BI, Wisch N, et al. Treatment of Crohn's disease with 6-mercaptopurine. A long-term, randomized, double-blind study. *N Engl J Med.* 1980; 302:981–987. [PubMed: 6102739]
12. Greenberg GR, Feagan BG, Martin F, et al. Oral budesonide for active Crohn's. disease Canadian Inflammatory Bowel Disease Study Group. *N Engl J Med.* 1994; 331:836–841. [PubMed: 8078529]
13. Sandborn WJ, Feagan BG, Marano C, et al. Subcutaneous golimumab induces clinical response and remission in patients with moderate-to-severe ulcerative colitis. *Gastroenterology.* 2014; 146:85–95. quiz e14–5. [PubMed: 23735746]
14. Panaccione R, Ghosh S, Middleton S, et al. Combination therapy with infliximab and azathioprine is superior to monotherapy with either agent in ulcerative colitis. *Gastroenterology.* 2014; 146:392–400. e3. [PubMed: 24512909]
15. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med.* 2010; 362:1383–1395. [PubMed: 20393175]
16. Dave M, Purohit T, Razonable R, et al. Opportunistic infections due to inflammatory bowel disease therapy. *Inflamm Bowel Dis.* 2014; 20:196–212. [PubMed: 24051931]
17. Singh S, Kumar N, Loftus EV Jr, et al. Neurologic complications in patients with inflammatory bowel disease: increasing relevance in the era of biologics. *Inflamm Bowel Dis.* 2013; 19:864–872. [PubMed: 22552994]
18. Dulai PS, Siegel CA. The Risk of Malignancy Associated with the Use of Biological Agents in Patients with Inflammatory Bowel Disease. *Gastroenterol Clin North Am.* 2014; 43:525–541. [PubMed: 25110257]
19. Armstrong L, Lako M, Buckley N, et al. Editorial: Our top 10 developments in stem cell biology over the last 30 years. *Stem Cells.* 2012; 30:2–9. [PubMed: 22162299]
20. Muller FJ, Laurent LC, Kostka D, et al. Regulatory networks define phenotypic classes of human stem cell lines. *Nature.* 2008; 455:401–405. [PubMed: 18724358]

21. Keller G. Embryonic stem cell differentiation: emergence of a new era in biology and medicine. *Genes Dev.* 2005; 19:1129–1155. [PubMed: 15905405]
22. Korbli M, Estrov Z. Adult stem cells for tissue repair - a new therapeutic concept? *N Engl J Med.* 2003; 349:570–582. [PubMed: 12904523]
23. Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. *Nat Rev Immunol.* 2006; 6:93–106. [PubMed: 16491134]
24. Copelan EA. Hematopoietic stem-cell transplantation. *N Engl J Med.* 2006; 354:1813–1826. [PubMed: 16641398]
25. Garcia-Bosch O, Ricart E, Panes J. Review article: stem cell therapies for inflammatory bowel disease - efficacy and safety. *Aliment Pharmacol Ther.* 2010; 32:939–952. [PubMed: 20804451]
26. Hawkey CJ. Stem cells as treatment in inflammatory bowel disease. *Dig Dis.* 2012; 30(Suppl 3): 134–139. [PubMed: 23295704]
27. Koo BK, Clevers H. Stem cells marked by the R-spondin receptor LGR5. *Gastroenterology.* 2014; 147:289–302. [PubMed: 24859206]
28. Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol.* 2007; 213:341–347. [PubMed: 17620285]
29. Kern S, Eichler H, Stoeve J, et al. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells.* 2006; 24:1294–1301. [PubMed: 16410387]
30. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science.* 1999; 284:143–147. [PubMed: 10102814]
31. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science.* 1997; 276:71–74. [PubMed: 9082988]
32. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells, The International Society for Cellular Therapy position statement. *Cytotherapy.* 2006; 8:315–317. [PubMed: 16923606]
33. Gonzalez-Rey E, Anderson P, Gonzalez MA, et al. Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. *Gut.* 2009; 58:929–939. [PubMed: 19136511]
34. Bartholomew A, Sturgeon C, Siatskas M, et al. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol.* 2002; 30:42–48. [PubMed: 11823036]
35. Di Nicola M, Carlo-Stella C, Magni M, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood.* 2002; 99:3838–3843. [PubMed: 11986244]
36. English K. Mechanisms of mesenchymal stromal cell immunomodulation. *Immunol Cell Biol.* 2013; 91:19–26. [PubMed: 23090487]
37. Liu H, Liu S, Li Y, et al. The role of SDF-1-CXCR4/CXCR7 axis in the therapeutic effects of hypoxia-preconditioned mesenchymal stem cells for renal ischemia/reperfusion injury. *PLoS One.* 2012; 7:e34608. [PubMed: 22511954]
38. Sasaki M, Abe R, Fujita Y, et al. Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. *J Immunol.* 2008; 180:2581–2587. [PubMed: 18250469]
39. Qiu Y, Marquez-Curtis LA, Janowska-Wieczorek A. Mesenchymal stromal cells derived from umbilical cord blood migrate in response to complement C1q. *Cytotherapy.* 2012; 14:285–295. [PubMed: 22264191]
40. Ponte AL, Marais E, Gallay N, et al. The in vitro migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. *Stem Cells.* 2007; 25:1737–1745. [PubMed: 17395768]
41. Ren G, Zhang L, Zhao X, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell.* 2008; 2:141–150. [PubMed: 18371435]

42. Krampera M, Cosmi L, Angeli R, et al. Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. *Stem Cells*. 2006; 24:386–398. [PubMed: 16123384]
43. Ge W, Jiang J, Arp J, et al. Regulatory T-cell generation and kidney allograft tolerance induced by mesenchymal stem cells associated with indoleamine 2,3-dioxygenase expression. *Transplantation*. 2010; 90:1312–1320. [PubMed: 21042238]
44. Brown JM, Nemeth K, Kushnir-Sukhov NM, et al. Bone marrow stromal cells inhibit mast cell function via a COX2-dependent mechanism. *Clin Exp Allergy*. 2011; 41:526–534. [PubMed: 21255158]
45. Anderson P, Souza-Moreira L, Morell M, et al. Adipose-derived mesenchymal stromal cells induce immunomodulatory macrophages which protect from experimental colitis and sepsis. *Gut*. 2013; 62:1131–1141. [PubMed: 22637701]
46. English K, Barry FP, Mahon BP. Murine mesenchymal stem cells suppress dendritic cell migration, maturation and antigen presentation. *Immunol Lett*. 2008; 115:50–58. [PubMed: 18022251]
47. Djouad F, Charbonnier LM, Bouffi C, et al. Mesenchymal stem cells inhibit the differentiation of dendritic cells through an interleukin-6-dependent mechanism. *Stem Cells*. 2007; 25:2025–2032. [PubMed: 17510220]
48. Nauta AJ, Kruisselbrink AB, Lurvink E, et al. Mesenchymal stem cells inhibit generation and function of both CD34+–derived and monocyte-derived dendritic cells. *J Immunol*. 2006; 177:2080–2087. [PubMed: 16887966]
49. Zhang B, Liu R, Shi D, et al. Mesenchymal stem cells induce mature dendritic cells into a novel Jagged-2-dependent regulatory dendritic cell population. *Blood*. 2009; 113:46–57. [PubMed: 18832657]
50. Li YP, Paczesny S, Lauret E, et al. Human mesenchymal stem cells license adult CD34+ hemopoietic progenitor cells to differentiate into regulatory dendritic cells through activation of the Notch pathway. *J Immunol*. 2008; 180:1598–1608. [PubMed: 18209056]
51. Akiyama K, Chen C, Wang D, et al. Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis. *Cell Stem Cell*. 2012; 10:544–555. [PubMed: 22542159]
52. Augello A, Tasso R, Negrini SM, et al. Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. *Eur J Immunol*. 2005; 35:1482–1490. [PubMed: 15827960]
53. Sioud M. New insights into mesenchymal stromal cell-mediated T-cell suppression through galectins. *Scand J Immunol*. 2011; 73:79–84. [PubMed: 21198747]
54. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med*. 2009; 151:264–269. W64. [PubMed: 19622511]
55. Higgins, JPTGSE. *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0 [updated March 2011]. Vol. 2014. The Cochrane Collaboration; 2011.
56. Leeflang MM, Deeks JJ, Gatsonis C, et al. Systematic reviews of diagnostic test accuracy. *Ann Intern Med*. 2008; 149:889–897. [PubMed: 19075208]
57. Freeman MTJW. Transformations related to the angular and the square root. *The Annals of Mathematical Statistics*. 1950; 21:607–611.
58. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986; 7:177–188. [PubMed: 3802833]
59. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *BMJ*. 2003; 327:557–560. [PubMed: 12958120]
60. Guyatt GH, Oxman AD, Kunz R, et al. GRADE guidelines: 7 Rating the quality of evidence--inconsistency. *J Clin Epidemiol*. 2011; 64:1294–1302. [PubMed: 21803546]
61. Team RC. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2013.
62. Schwarzer G. meta: General Package for Meta-Analysis. R package version 4.1–0. 2015

63. Herreros MD, Garcia-Arranz M, Guadalajara H, et al. 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; 55:762–772. [PubMed: 22706128]
64. Garcia-Olmo D, Garcia-Arranz M, Garcia LG, et al. Autologous stem cell transplantation for treatment of rectovaginal fistula in perianal Crohn's disease: a new cell-based therapy. *Int J Colorectal Dis*. 2003; 18:451–454. [PubMed: 12756590]
65. Garcia-Olmo D, Garcia-Arranz M, Herreros D, et al. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Dis Colon Rectum*. 2005; 48:1416–1423. [PubMed: 15933795]
66. Cirocchi R, Farinella E, La Mura F, et al. Fibrin glue in the treatment of anal fistula: a systematic review. *Ann Surg Innov Res*. 2009; 3:12. [PubMed: 19912660]
67. Cirocchi R, Santoro A, Trastulli S, et al. Meta-analysis of fibrin glue versus surgery for treatment of fistula-in-ano. *Ann Ital Chir*. 2010; 81:349–356. [PubMed: 21294388]
68. Garcia-Olmo D, Herreros D, Pascual I, et al. Expanded adipose-derived stem cells for the treatment of complex perianal fistula: a phase II clinical trial. *Dis Colon Rectum*. 2009; 52:79–86. [PubMed: 19273960]
69. Ciccocioppo R, Bernardo ME, Sgarella A, et al. Autologous bone marrow-derived mesenchymal stromal cells in the treatment of fistulising Crohn's disease. *Gut*. 2011; 60:788–798. [PubMed: 21257987]
70. de la Portilla F, Alba F, Garcia-Olmo D, et al. 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*. 2013; 28:313–323. [PubMed: 23053677]
71. Cho YB, Lee WY, Park KJ, et al. Autologous adipose tissue-derived stem cells for the treatment of Crohn's fistula: a phase I clinical study. *Cell Transplant*. 2013; 22:279–285. [PubMed: 23006344]
72. Lee WY, Park KJ, Cho YB, et al. Autologous adipose tissue-derived stem cells treatment demonstrated favorable and sustainable therapeutic effect for Crohn's fistula. *Stem Cells*. 2013; 31:2575–2581. [PubMed: 23404825]
73. Cho YB, Park KJ, Yoon SN, et al. Long-Term Results of Adipose-Derived Stem Cell Therapy for the Treatment of Crohn's Fistula. *Stem Cells Transl Med*. 2015
74. Van Assche G, Dignass A, Reinisch W, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Special situations. *J Crohns Colitis*. 2010; 4:63–101. [PubMed: 21122490]
75. Onken JGD, Hanson J, Pandak M, Custer L. Successful outpatient treatment of refractory Crohn's disease using adult mesenchymal stem cells. *American College of Gastroenterology Annual Meeting*. 2006
76. Duijvestein M, Vos AC, Roelofs H, et al. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: results of a phase I study. *Gut*. 2010; 59:1662–1669. [PubMed: 20921206]
77. Lazebnik LB, Konopliannikov AG, Kniazev OV, et al. [Use of allogeneic mesenchymal stem cells in the treatment of intestinal inflammatory diseases]. *Ter Arkh*. 2010; 82:38–43. [PubMed: 20387674]
78. Liang J, Zhang H, Wang D, et al. Allogeneic mesenchymal stem cell transplantation in seven patients with refractory inflammatory bowel disease. *Gut*. 2012; 61:468–469. [PubMed: 21617158]
79. Forbes GM, Sturm MJ, Leong RW, et al. A phase 2 study of allogeneic mesenchymal stromal cells for luminal Crohn's disease refractory to biologic therapy. *Clin Gastroenterol Hepatol*. 2014; 12:64–71. [PubMed: 23872668]
80. Martin PJJP, Soiffer RJ, Klingemann H, Waller EK, Daly AS, Herrmann RP, Kebriaei P. Prochymal improves response rates in patients with steroid-refractory acute graft versus host disease (SR-GVHD) involving the liver and gut: Results of a randomized, placebo-controlled, multicenter phase III trial in GVHD. *Biol Blood Marrow Transplant*. 2010; 16:169–170.

81. Galipeau J. The mesenchymal stromal cells dilemma--does a negative phase III trial of random donor mesenchymal stromal cells in steroid-resistant graft-versus-host disease represent a death knell or a bump in the road? *Cytotherapy*. 2013; 15:2–8. [PubMed: 23260081]
82. Duijvestein M, Wildenberg ME, Welling MM, et al. Pretreatment with interferon-gamma enhances the therapeutic activity of mesenchymal stromal cells in animal models of colitis. *Stem Cells*. 2011; 29:1549–1558. [PubMed: 21898680]
83. Kim HS, Shin TH, Lee BC, et al. Human umbilical cord blood mesenchymal stem cells reduce colitis in mice by activating NOD2 signaling to COX2. *Gastroenterology*. 2013; 145:1392–1403. e1–e8. [PubMed: 23973922]
84. Ankrum JA, Dastidar RG, Ong JF, et al. Performance-enhanced mesenchymal stem cells via intracellular delivery of steroids. *Sci Rep*. 2014; 4:4645. [PubMed: 24717973]
85. Dave M, Hayashi Y, Gajdos GB, et al. Stem Cells for Murine Interstitial Cells of Cajal Suppress Cellular Immunity and Colitis Via Prostaglandin E Secretion. *Gastroenterology*. 2015; 148(5): 978–990. [PubMed: 25637652]

Figure 1.
Different mechanisms of immunoregulation by mesenchymal stem cells

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Cho et al 2012			-	-	+	-	
Ciccocioppo et al 2011			-	+	+	+	
de la Portilla et al 2013			-	+	-	+	
Duijvestein et al 2010			-	-	-	+	
Forbes et al 2013			-	-	+	+	
García-Olmo et al 2003			-	-	+	-	
García-Olmo et al 2005			-	-	-	-	
García-Olmo et al 2009	+	+	-	+	+	-	
Lazebnik et al 2010	-	-	-	-	+	+	
Lee et al 2013			-	-	-	-	
Liang et al 2012			-	-	+	-	
Onken et al 2006			-	-	+	-	

Figure 2.

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.

Plus sign denotes low risk. Minus sign denotes high risk. Blank denotes unclear risk.

Figure 3.

Forrest plot of studies evaluating healing of peri-anal fistulas (peri-anal CD) after local administration/injection of MSCs.

Figure 4.
 Forrest plot of studies evaluating induction of clinical remission after systemic administration of MSCs.

Table 1

Stem cell therapy by local injection for perianal fistulizing Crohn's disease

Name of Study	Type of Study	Location	CD	Intervention	Type and source of Stem Cells	Outcome	Results	Use of MRI
García-Olmo et al 2003	Case report	Spain	1	Local injection of stem cells	Autologous, Adipose tissue	Reepithelialization of external opening of fistula	Fistula healed in 1 week. No recurrence till 3 months post treatment	No
García-Olmo et al 2005	Phase I, open label, single arm	Spain	5	Local injection of stem cells	Autologous, Adipose tissue	Reepithelialization of external opening of fistula	3 out of 4 rectovaginal or perianal fistula (75%) and 3 out of 4 enterocutaneous fistulas (75%) healed at eight weeks post treatment.	No
García-Olmo et al 2009	Phase II, open label, double arm, randomized	Spain	14	Local injection of stem cells plus fibrin glue as compared to fibrin glue alone	Autologous, Adipose tissue	Reepithelialization of external opening of fistula	5 out of 7 fistulas (71%) healed in stem cells plus fibrin glue group as compared to 1 out of 7 fistulas (14%) healed in fibrin glue alone group after 8 weeks of treatment	No
Ciccocioppo et al 2011	Open label, single arm	Italy	10	Local injection of stem cells	Autologous, Bone marrow	Complete closure evident on clinical exam as well as MRI	One patient with multiple enterocutaneous and 6 out of 9 patients (67%) with complex perianal fistula had complete closure and other 3 had incomplete closure at 8 weeks post treatment	Yes
de la Portilla et al 2013	Phase I/IIa open label, single arm	Spain	24	Local injection of stem cells	Allogeneic, Adipose tissue	Fistula closure was defined as: absence of suppuration through the external orifice and complete re-epithelialization, plus absence of collections measured by MRI	5 out of 18 fistulas (28%) closed at 24 weeks post treatment. 7 out of 18 patients (47%) had closure of external openings at 24 weeks post treatment.	Yes
Cho et al 2012	Phase I, open label, single arm	Korea	10	Local injection of stem cells	Autologous, Adipose tissue	Healing of fistula	3 out of 10 patients (30%) had complete healing of fistula at 8 weeks post treatment.	No
Lee et al 2013	Phase II, open label, single arm	Korea	43	Local injection of stem cells	Autologous, Adipose tissue	Healing of fistula	27 out of 33 patients (82%) had complete fistula healing at 8 weeks post treatment	No

CD: Crohn's Disease

Table 2

Stem cell therapy by systemic infusion for inflammatory bowel disease

Name of Study	Type of Study	Location	UC	CD	Intervention	Type and source of Stem Cells	Outcome	Results
Onken et al 2006	Phase II, open label, double arm, randomized	USA	0	10	Systemic infusion of low vs. high dose stem cells	Allogeneic, Bone marrow	CDAI	<ul style="list-style-type: none"> 3 out of 9 patients showed clinical response (drop in CDAI > 100) at day 14 post treatment. Induction of remission (CDAI < 150) in 1 out of 9 patients by day 7 post treatment.
Duijvestein et al 2010	Phase I, open label, single arm	Netherlands	0	10	Systemic infusion of stem cells	Autologous, Bone marrow	CDAI and CDEIS	<ul style="list-style-type: none"> 3 out of 10 patients showed clinical response (drop in CDAI > 70) at 6 weeks post treatment. None achieved remission (CDAI < 150). CDEIS improved in 2 patients (by 10 and 24.7 points)
Lazebnik et al 2010	Open label, double arm	Russia	69	21	Systemic infusion of stem cells (39 UC and 11 CD patients) vs. Traditional treatment (30 UC and 10 CD patients)	Allogeneic, Bone marrow	CDAI, RCAI, Mayo and Gebs scales	<ul style="list-style-type: none"> A statistically significant decrease in the indices of the clinical and morphological activities of an inflammatory process was noted in 39 patients with UC and in 11 patients with CD as compared with the comparison groups after MSC transplantation. Clinico-morphological remission occurred in 40 patients (80%). 34 of 50 patients (68%) were able to taper off steroids after MSCs administration.
Liang et al 2012	n/a	China	3	4	Systemic infusion of stem cells	Allogeneic, Bone marrow and Umbilical Cord	CDAI, CAI	<ul style="list-style-type: none"> All 7 patients had significant reduction in CDAI and CAI after treatment. Five patients achieved remission at 3 months post treatment. The maintenance of remission lasted for 24 months in 2 patients
Forbes et al 2013	Phase II, open label, single arm.	Australia	0	16	Systemic infusion of stem cells	Allogeneic, Bone marrow	CDAI, CDEIS	<ul style="list-style-type: none"> CDAI decreased by > 100 in 12 of 15 patients (80%) at 42 days post treatment Clinical remission (CDAI < 150) in 8 of 15 patients at 42 days post treatment Mean CDEIS scores decreased from 21.5 (range, 3.3–33) to 11.0 (range, 0.3–18.5) in 7 of 15 patients (47%).

UC: Ulcerative Colitis, CD: Crohn's Disease, CDAI: Crohn's Disease Activity Index, CDEIS: Crohn's Disease Endoscopic Index of Severity, RCAI: Rachmilewicz clinical activity index

Table 3

Adverse events of stem cell therapy by local injection for fistulizing Crohn's disease

Adverse Event	Crohn's Disease Patients with SCT Therapy (N = 104)	Non IBD patients with SCT Therapy (N = 17)	Crohn's Disease Patients without SCT Therapy (N = 7)
Perianal infection	8	3	?
Perianal abscess	4	1	1
Intraabdominal abscess	0	0	1
Proctalgia	4	0	0
Cholecystitis	0	1	0
Fever	3	1	1
Anxiety	3	0	0
Diarrhea	2	0	0
Uterine leiomyoma	1	0	0
Allergic reaction	0	0	0
Flare of Crohn's disease	0	0	1
Malignancy	0	0	0
Death	0	0	0

Table 4

Adverse events of stem cell therapy by systemic infusion for inflammatory bowel disease

Adverse Event	Number of patients affected (N = 93)
Taste or smell disturbances	25
Fever	13
Myalgia	12
Arthralgia	12
Headache	6
Increased ALT	4
Allergic reaction	3
Abdominal pain	3
Anorexia	2
Fatigue	2
Flare of Crohn's disease	2
Lymphopenia	2
Nausea	2
Anemia	1
Common cold	1
Diarrhea	1
Dizziness	1
Insomnia	1
Neoplasia	1
Otitis media	1
Vaginal candidiasis	1
Viral gastroenteritis	1
Vomiting	1
Anxiety	0
Death	0
Perianal abscess	0
Perianal infection	0
Proctalgia	0
Uterine leiomyoma	0