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Review article: mesenchymal stromal cell therapy for inflammatory bowel diseases

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SUMMARY

Background
Inflammatory bowel diseases (IBD) are chronic relapsing diseases in which pro-inflammatory immune cells and cytokines induce intestinal tissue damage and disability. Mesenchymal stromal cells (MSCs) exert powerful immunomodulatory effects and stimulate tissue repair.

Aim
To review the current data on mesenchymal stromal cell therapy in IBD.

Method
We searched PubMed and 'ClinicalTrials.gov' databases using the terms 'mesenchymal stromal cells', 'mesenchymal stem cell transplantation', 'inflammatory bowel diseases', 'Crohn disease' and 'colitis, ulcerative'. Additional publications were identified from individual article reference lists.

Results
MSCs include inhibition of Th1/Th17 lymphocytes and recruitment of regulatory T lymphocytes, induction of antigen-presenting cells into a regulatory-like profile, and stimulation of epithelial cell differentiation and proliferation. More than 200 patients with refractory fistulas have been treated with local injections of MSCs, resulting in complete response in more than half, and in overall response in approximately two thirds of patients. In refractory luminal Crohn's disease, 49 cases of systemic MSC infusions have been reported, while trials with autologous MSCs resulted in mitigated responses, studies using allogeneic MSCs were promising, with around 60% of patients experiencing a response and around 40% achieving clinical remission.

Conclusions
Mesenchymal stromal cells might represent a promising therapy for IBD, especially for Crohn's disease. There remain many unsolved questions concerning the optimal origin and source of mesenchymal stromal cells, dosage and modalities of administration. Moreover, mesenchymal stromal cells still need to prove their effectiveness compared with conventional treatments in randomised controlled trials.

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INTRODUCTION

Inflammatory bowel diseases (IBD) are multifactorial chronic relapsing diseases involving an abnormal systemic and mucosal immune response against intraluminal antigens, favoured by microbial factors and alteration of the mucosal barrier.[1, 2] A loss of balance between proinflammatory T helper (Th)1/Th17 cells and regulatory T lymphocytes (Treg), resulting in the activation of macrophages and B cells as well as recruitment of circulating leucocytes into the gut, seems to play a key role in the pathogenesis of IBD.

Mesenchymal stromal cells (MSCs) are multipotent progenitors that can be isolated from the connective tissues of most organs. In addition to multilineage differentiation and participation in the haematopoietic niche, they exert powerful immunomodulatory effects, including inhibition of proliferation and function of Th1 and Th17 cells, and promotion of Treg differentiation. Moreover, they display several properties that could make them interesting for tissue repair: homing to sites of inflammation and tissue damage, differentiation into various cell types, secretion of soluble factors stimulating survival and recovery of injured cells and tissues (Figure 1). The relative importance of these properties for the efficacy of MSCs on experimental colitis and IBD is still investigated.

METHODS

Bibliographic searches were performed in PubMed using the search terms 'mesenchymal stromal cells', 'mesenchymal stem cell transplantation', 'inflammatory bowel diseases', 'Crohn disease' and 'colitis, ulcerative'. The articles were selected based on relevance to this review. We included additional publications that were identified through review of individual article reference lists. A systematic search was also performed on 'ClinicalTrials.gov' using the search terms 'mesenchymal stromal cells', 'inflammatory bowel diseases', 'Crohn's disease' and 'ulcerative colitis'.

PROPERTIES OF MSCS

Mesenchymal stromal cells can be isolated from bone marrow, where they support haematopoiesis, but also from various other tissues such as umbilical cord, umbilical cord blood, placenta, adipose tissue, and seem to reside in the connective tissues of most organs.[3, 4] The three minimal criteria to define MSCs are as follows: (i) adhesion to plastic in standard culture conditions,

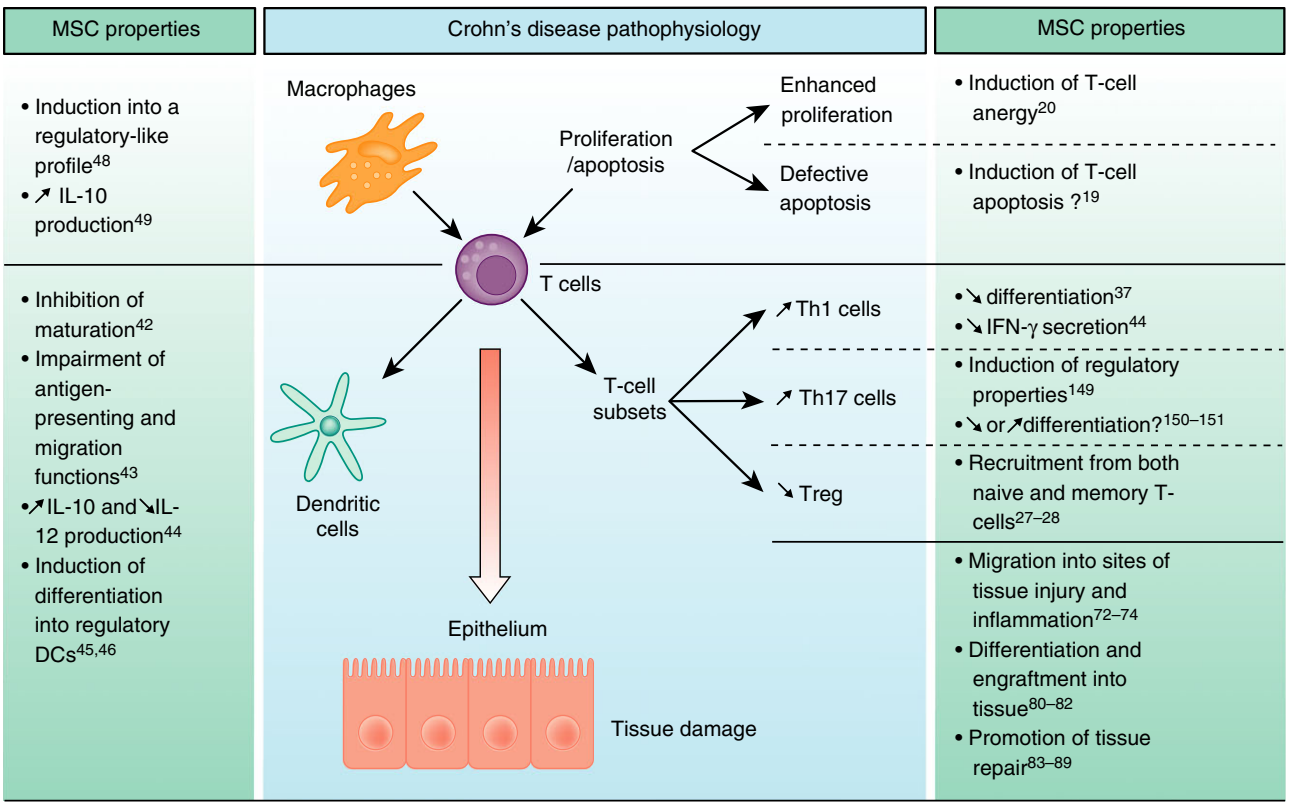

Figure 1 | Potential therapeutic effects of mesenchymal stromal cell in Crohn's disease.

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(ii) expression of CD73, CD90 and CD105, and lack of expression of CD34, CD45, CD11b or CD14, CD19 or CD79a and human leucocyte antigen (HLA)-DR surface molecules, and (iii) *in vitro* differentiation into osteoblasts, chondroblasts and adipocytes.[5] MSCs are considered as immunoprivileged because they express low levels of HLA class I molecules and do not express HLA class II nor co-stimulatory molecules (CD80, CD86 or CD40) under normal circumstances.[6, 7]

Immunomodulation

Mesenchymal stromal cells immunomodulatory effects are pleiotropic and not yet fully understood. They probably vary among species[8] and depend on many parameters such as culture conditions or an inflammatory environment. MSCs were first demonstrated to inhibit T-cell proliferation, whether the lymphocytes are $CD4^+$ or $CD8^+$, naive or memory, and regardless of their functional state,[9–13] and independently of the major histocompatibility complex, as inhibition is similar using 'third party' or autologous MSC.[9] The effect of MSCs on T cells seems to depend on the MSC/T-cell ratio: a high MSC/T-cell ratio exerts strong inhibitory effects, whereas a low MSC/T-cell ratio might enhance T-cell proliferation.[14]

A key role in the pathogenesis of IBD seems to be played by enhanced proliferation and defective apoptosis of immune cells (attributed to an imbalance of the anti-apoptotic protein Bcl-2 and the proapoptotic protein Bax),[15–17] and it has been demonstrated that Fas-mediated apoptosis is lower in Crohn's disease than in control T cells.[18] Whether MSC-inhibited T cells undergo apoptosis or not has still to be resolved. A recent study found that systemic infusion of murine bone marrow MSCs induced T-cell apoptosis through the Fas ligand-/Fas pathway, and that FASL-/- bone marrow MSCs were ineffective in a murine model of dextran sodium sulphate (DSS)-induced colitis.[19] However, *in vitro*, some authors observed that MSCs did not induce T-cell apoptosis,[11] but rather blocked them in G0/G1 phase of the cell cycle, in a state of anergy, by inhibiting cyclin D2 expression,[20] or even prevented activation-induced cell death through downregulation of Fas receptor and Fas ligand on TCR-activated T cells.[21]

The pathogenesis of IBD also involves an imbalance in T-cell subsets, with proinflammatory cytokines arising from Th1 and Th17 cell differentiation in Crohn's disease, and Th2 cell differentiation in ulcerative colitis,[22–24] whereas Treg is down-regulated.[25] Among Treg cells, $CD4^+CD25^+FoxP3^+$ T cells play a key role in suppressing the immune system and maintaining self-tolerance, through inhibition of proinflammatory cytokine production, downregulation of costimulatory molecules on antigen presenting cells, and modulation of T-cell proliferation and differentiation.[26] Treg levels are lower in peripheral blood in IBD patients (both ulcerative colitis and Crohn's disease) than in healthy controls.[25] Recent studies suggest that an important part of MSC-mediated immunomodulation results from the recruitment of Treg from both naive and memory T cells.[27, 28] The mechanisms involve secretion of soluble factors, such as prostaglandin (PG)-E2, transforming growth factor (TGF)-β1 or HLA-G, interaction between CCL1 and CCR8 on T cells, and induction of immature dendritic cells and monocytes into a regulatory profile.[29–32] *In vivo*, Treg expansion after MSC infusion has been observed in several dysimmune diseases.[33–35] In several models of DSS- and trinitrobenzene sulphonate (TNBS)-induced colitis in mice treated with intravenous or intraperitoneal injection of human adipose tissue-derived, umbilical cord or bone marrow MSCs, MSCs successfully treated colitis (clinical response and decreased infiltration of inflammatory cells in the lamina propria), and authors mostly observed a decreased number of interferon (IFN)-γ-producing Th1 cells and higher numbers of Treg cells in mesenteric lymph nodes and colon, lower systemic levels of Th1 and Th17 pro-inflammatory cytokines [including tumour necrosis factor (TNF)-α, IFN-γ, interleukin (IL)-6, IL-12, IL-17, IL-23], and higher systemic levels of the anti-inflammatory cytokine IL-10.[36–39] In a murine model of TNBS-induced colitis, MSCs co-localised with $CD11b^+$ cells in the spleen, and these cells were essential to the expansion of Treg cells in draining mesenteric lymph nodes.[40]

Antigen presenting cells, such as dendritic cells and macrophages, are also involved in IBD pathogenesis, through generation and polarisation of T-cell responses.[41] *In vitro*, MSCs inhibit dendritic cell maturation (through a down-regulation of cyclin D2 and $p27^{Kip1}$ expression, resulting in a blockade in G0/G1 phase of the cell cycle), impair their antigen-presenting and migration functions, modulate their secretion of cytokines (increased IL-10 and decreased IL-12 production), and induce them to differentiate into regulatory dendritic cells (through the Notch signalling pathway).[42–46] These effects have been confirmed *in vivo* in murine models.[47] MSCs also interact with macrophages, inducing them into a regulatory-like profile[48] and increasing their production of IL-10.[49] In DSS and TNBS-induced mouse colitis, intraperitoneal injection of

murine bone marrow macrophages cultured with adipose tissue-derived MSCs successfully treated colitis and sepsis. *In vitro*, these macrophages displayed a regulatory phenotype [high arginase activity, increased expression of IL-10 and inducible NO synthase (iNOS), lower production of TNFα and IL-12] and showed potent immunosuppressive activity on splenocytes.[50]

Effects of MSCs on B cells and natural killer cells are still debated and may depend on environmental signals and MSC/effector cell ratio. Most studies found that MSCs inhibit B-cell activation, proliferation, chemotaxis and IgG secretion, and it was recently demonstrated that MSCs could increase IL-10 producing $CD5^+$ regulatory B cells (Bregs),[51–54] but one study found that MSC promoted proliferation and differentiation of naive B cells into immunoglobulin-secreting cells.[55] Similarly, MSCs seem to inhibit natural killer cell proliferation and their secretion of pro-inflammatory cytokines, but whether they do or do not decrease their cytotoxic activity is controversial.[30, 56–59]

Mechanisms of MSC-mediated immunomodulation *in vitro* imply direct cell contacts and secretion of soluble factors, mostly PGE2, indoleamine 2,3 dioxygenase (IDO), nitric oxide (NO) and HLA-G.[29, 30, 60–62] In DSS or TNBS-induced mouse colitis, NOD2-activated human umbilical cord MSCs reduced the severity of colitis through the secretion of PGE-2, and this was associated with increased Treg colonic infiltration and increased IL-10 levels.[63] IL-10 is probably not secreted directly by MSCs but participate to their immunosuppressive effect, as this effect is decreased by IL-10 blockade.[35] The transcription factor Aire might also be involved in MSC immunomodulation through repression of early T-cell activation factor 1 (Eta-1), as it was required for their efficacy in a model of chronic colitis (induced by transfer of $CD4^+$ CD45RBhi T cells in Rag−/− mice), but it did not control MSC suppression of T-cell proliferation *in vitro*.[64]

As mentioned above, immunomodulatory effects of MSCs depend on the inflammatory environment. Many factors have been shown to modulate MSC-mediated immunosuppression, such as toll-like receptors (TLRs),[65–67] IFN-γ, TNF-α and IL-1β.[68] IFN-γ pretreated human bone marrow MSCs or IFN-γ-transfected umbilical cord MSCs were more potent to inhibit peripheral blood mononuclear cell or T-lymphocyte proliferation *in vitro*, and were superior to resting MSCs to treat DSS- and/or TNBS-induced colitis. This was associated with an increased expression of MHC II, IDO and iNOS, lower expression of several colonic inflammatory cytokines (such as IL-6, IL-1β, TNF-α, IFN-γ or IL-17A). An increased percentage of Treg and Th2 cells and a decreased percentage of Th1 and Th17 cells have also been observed both in mesenteric lymph nodes and in spleen of mice treated with IFN-γ primed MSC.[69, 70] IL-1β-priming of human umbilical cord MSCs increases their expression of COX-2, IL-6 and IL-8 mRNA and improves their ability to migrate into the spleen, mesenteric lymph nodes and colon (through upregulation of CXCR4 expression), and to attenuate DSS-induced murine colitis (associated with increased numbers of peritoneal M2 macrophages and splenic or mesenteric lymph node Treg).[71]

Tissue regeneration

Homing. Mesenchymal stromal cells have demonstrated their ability to migrate selectively into various sites of tissue injury and inflammation,[72–74] including inflamed zones of the intestine (lamina propria, muscular layer and submucosa) and mesenteric lymph nodes in murine models of colitis, whatever their origin or their route of administration.[37–39, 74–76] We still lack information on the persistence of MSCs in these tissues since, in most studies, MSC tracking analyses were only performed during the first week after MSC injection, and MSCs were detected in inflamed zones of the intestine every time,[74–76] except in one study where MSCs were detected until day 5, but not on day 7[37]; however, MSCs were still found in inflamed colonic tissues 15 days after injection in the only study in which tracking analyses were performed until that time point.[38] Signals modulating MSC migration include growth factors [vascular endothelial growth factor (VEGF), platelet-derived growth factor, etc.],[77, 78] and interaction between chemokines and various C-C chemokine receptors.[35] Moreover, MSCs express different adhesion molecules involved in tissue transmigration [intercellular adhesion molecule (ICAM), vascular cell adhesion protein (VCAM), etc.] and integrins.[36] Activation by IFN-γ or IL-1β and targeting via VCAM antibody-coating may improve the homing of MSCs into mesenteric lymph nodes and colon, and therefore their efficacy on murine colitis.[69, 70, 79]

Differentiation and tissue repair stimulation. In response to inflammation or tissue injury, MSCs are able to differentiate into various cells of the mesenchymal lineage and to engraft into many tissues,[80–82] where they promote tissue repair.[83–89] In a model of DSS-induced rat colitis, intravenously administered rat MSCs migrated to the lamina propria of the injured colon, where they

upregulated their expression of α-smooth muscle actin (α-SMA), and were able to heal epithelial injuries. However, tissue repair properties of MSCs are not so much due to their differentiation capacities, but rather to their ability to stimulate survival and recovery of local tissues, through stimulation of angiogenesis and inhibition of apoptosis and fibrosis in injured tissues.[90–92] In mice suffering from TNBS-induced colitis, intravenous injection of murine bone marrow MSC increased proliferation of intestinal epithelial cells and differentiation of intestinal stem cells, as attested by the increased expression of Ki67 and leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5) in damaged tissues.[75] In a rat model of TNBS-induced colitis, topically implanted rat bone marrow MSCs were detectable in submucosal layers at days 3 and 6 post-implantation and successfully healed mucosal injuries. A small proportion of MSCs was positive for α-SMA and desmin, indicating differentiation into myofibroblasts, but most rather expressed VEGF and TGF-β1, two important growth factors in gastrointestinal wound healing.[93] In DSS-induced colitis in rats, intravenously administered allogeneic MSCs reduced epithelial injury and restored expression of claudin-2, claudin-12 and claudin-15, proteins constituting tight junctions and known to have a major role in the paracellular permeability of the epithelial barrier.[94]

MSC THERAPY IN IBD

Autologous and allogeneic MSCs have been evaluated in human trials, in two different modalities: local injection of MSCs to treat fistulising Crohn's disease and intravenous injection of MSCs to treat luminal Crohn's disease or ulcerative colitis.

MSC therapy for fistulising Crohn's disease

Management of fistulas associated with Crohn's disease has remained a challenging problem since many such fistulas do not respond to available treatments, including most effective molecules such as anti-TNF. Surgical treatments (i.e. endoanal advancement flap) can cure some fistulas but anal incontinence and recurrence are not rare. Local administration of autologous or allogeneic adipose tissue-derived or bone marrow MSCs have suggested some efficacy and reassuring safety in several phase I or II trial. Results were uniformly positive whatever the origin and despite variation in dose and schema of injection, and two randomised controlled trials demonstrated the superiority of autologous and allogeneic MSCs over placebo (Table 1). To date, more than 200 patients with refractory fistulas associated with Crohn's disease have been treated with local injections of MSCs, resulting in complete response in more than half (sustained for at least 24 weeks for around 75% of them) and in overall response in approximately two thirds of patients. Several phase I–III clinical trials aimed at further exploring local injection of autologous or allogeneic adipose tissue-derived or bone marrow MSCs in fistulising Crohn's disease are currently ongoing (Table 2).

Autologous MSC therapy for fistulising Crohn's disease. In a phase I study, four patients suffering from one or more refractory complex Crohn's fistulas were treated with a single intrafistular injection of 3–30 $\times$ 10^6 autologous adipose tissue-derived MSCs. The authors observed healing of 6/8 fistulas at 8 weeks, and partial closure with decreased drainage in 2/8 fistulas. There were no adverse effects after 12- to 22-month follow-up.[95] Then, in a phase IIb trial, 49 patients with complex perianal fistulas were randomly assigned to treatment with intralesional injection of fibrin glue or fibrin glue plus 20 million adipose tissue-derived MSCs, followed by a second double dose of adipose tissue-derived MSCs if fistula healing was not obtained at 8 weeks. Fistula healing occurred in 17/24 patients receiving adipose tissue-derived MSCs (11 patients healed after the first injection and 6 patients after a second injection) compared with 4/25 in the control group (RR 4.43, $P < 0.001$). Improvement in quality of life was significantly greater in the treated group compared with the control group, even in patients whose fistula did not heal.[96] After a mean follow-up of 40 months, 7/12 patients treated with adipose tissue-derived MSCs remained free of recurrence (2/3 in the control group). The incidence of perianal sepsis was lower in the treated group ($P = 0.04$). No adverse effect was related to adipose tissue-derived MSCs, confirming their very good safety and tolerability profile.[97]

Another group performed a small dose escalation phase I study, in which 10 patients with a perianal fistula associated with Crohn's disease were treated with intrafistular injection of autologous adipose tissue-derived MSCs at a dose of 1 $\times$ 10^7, 2 $\times$ 10^7 or 4 $\times$ 10^7 cells/mL in proportion to the size of the fistula (total of 3–40 $\times$ 10^7 cells). After 8 weeks, complete healing was observed in 3/10 patients (2 of the 2 $\times$ 10^7 group and 1 of the 4 $\times$ 10^7 group), while partial closure with no drainage was observed in all other patients. These results were sustained after 8 months and no adverse effect was observed.[98] Then, the same group led a phase II trial, with 43 patients with perianal fistulae receiving a first intrafistular injection of adipose tissue-derived MSCs

Table 1 | Completed trials – intrafistular injection(s) of MSCs for fistulising Crohn's disease

Origin	Phase	*n*	Schema	Evaluation	Response
Autologous MSC therapy					
Adipose tissue	I	4	3–30 × 10^6 MSCs	Clinical evaluation at week 8	6/8 fistulas healed, 2/8 improved[95]
Adipose tissue	IIb	49	20 × 10^6 MSCs with fibrin glue or placebo (fibrin glue alone); repeated with 40 × 10^6 MSCs if incomplete closure at week 8	Clinical evaluation at week 8; 1-year follow-up	(i) Fistula healing in 17/24 patients, sustained in 7/12 patients (vs. 4/25 and 2/3 in control group) (ii) Improved quality of life (iii) Lower incidence of perianal sepsis[96, 97]
Adipose tissue	I	10	10, 20 or 40 × 10^6 MSCs/ml in proportion to the size of the fistula	Clinical evaluation at week 8; 8-month follow-up	Sustained complete healing in 3/7 patients, partial closure in all other patients[98]
Adipose tissue	II	43	30–60 × 10^6 MSCs/cm, with fibrin glue, repeated with 1.5 times more cells if incomplete closure at week 8	Clinical evaluation at week 8; 1-year follow-up	Complete fistula healing in 27/42 patients (sustained in 23/26 patients) and incomplete closure in 6/42 patients[99]
Bone marrow	I	10	2–5 monthly injections of 15–30 × 10^6 MSCs	Clinical, surgical and MRI evaluation at the time of each treatment and 3, 6 and 12 months after the last procedure; 1-year follow-up	(i) Complete closure of fistula tract in 7/10 patients, incomplete closure in 3/10 (ii) Sustained reduction of CDAI and PDAI in all patients (iii) Rectal mucosal healing in 7/7 patients. Increase in the percentage of circulating and mucosal Treg (at 12 months)[100] (iv) Increase in the percentage of circulating and mucosal Treg (at 12 months)[100]
Allogeneic MSC therapy					
Adipose tissue	I/IIa	24	20 × 10^6 MSCs, then 40 × 10^6 MSCs at week 12 if incomplete closure	Clinical and MRI evaluation at weeks 12 and 24; 24-week follow-up	(i) At week 12, improvement of ≥1 fistula in 12/20 patients (9/13 at week 24) and complete closure in 8/21 patients (9/16 at week 24) (ii) Decreased MRI Score of Severity at week 12 and PDAI at week 24; no effect on CDAI[101]
Adipose tissue	III	212	120 × 10^6 MSCs to all fistula tract or placebo	Clinical evaluation at weeks 6, 12, 18 and 24 and combined clinical and MRI evaluation at week 24	(i) Fistula closure in 53/107 patients in the MSC group (vs. 36/105 in the placebo group; P = 0.024), obtained in a significantly shorter time in the MSC group (6.7 vs. 14.6 weeks) (ii) Significantly greater improvement in PDAI in the MSC group vs. placebo at week 6, but not at week 24. Treatment-related adverse events in 18/103 patients in the MSC group vs. 30/103 in the placebo group[102]
Bone marrow	IIa	21	10, 30 or 90 × 10^6 MSCs, or placebo	Clinical evaluation at weeks 6, 12 and 24; endoscopic and MRI evaluation at week 12	(i) Healing in 8/15 patients (12/23 individual fistulas) in MSC groups at week 6 and 7/15 patients (11/23 fistulas) at week 12, vs. 1/6 and 2/6 patients (2/9 and 3/9 fistulas) in the placebo group, sustained at week 24 (ii) Better results in the 10 × 10^6 and 30 × 10^6 MSC groups compared to the 90 × 10^6 MSC group[103]

Table 2 | Ongoing protocols – Intrafistular injection(s) of MSC for fistulising Crohn's disease

NCT number	Localisation	Phase	Origin	Schema	Status
Autologous MSC therapy					
NCT 01874015	Royan Institute, Iran	I	Bone marrow	4 monthly injections	Recruiting
NCT 01915927	Mayo Clinic, USA	I	Adipose tissue	1 fistula plug coated with 20 × 10^6 MSCs	Recruiting
NCT 02403232	Papa Giovanni XXIII Hospital, Italy	II	Adipose tissue	1 injection	Recruiting
Allogeneic MSC therapy					
NCT 02677350	University of Miami Miller School of Medicine, USA	I	Bone marrow	1 injection of 20 × 10^7 MSCs, repeated maximum 4 times at 4-week intervals	Not yet recruiting
NCT 01541579	TiGenix, USA	III	Adipose tissue	1 injection of either 120 × 10^6 MSCs or placebo	Active, not recruiting

with a number of cells proportionate to the size of the fistula (3 × 10^7 or 6 × 10^7 cells per 1 cm of fistula length, depending on whether the diameter was less or more than 1 cm, average number of 15.8 × 10^7 cells), followed by a second injection of 1.5 times more cells (average number of 19.1 × 10^7 cells) if fistula closure was not complete at 8 weeks. Moreover, the fistula tract was filled with a mixture of adipose tissue-derived MSCs and fibrin glue. Complete fistula healing was observed in 27/42 patients by 8 weeks after the final adipose tissue-derived MSC injection and sustained after 1 year in 23/26 patients. Six other patients had an incomplete closure, with 5 of them achieving a closure by more than 50% of fistula tract with a decrease in drainage of more than 50%. No adverse event related to adipose tissue-derived MSCs was observed.[99]

Autologous bone marrow MSCs were evaluated in only one trial, with 10 patients suffering from fistulising Crohn's disease refractory to all previous medical treatments (including anti-TNF therapy) or surgery. They received intrafistular injections of 15–30 × 10^6 MSC every 4 weeks until an improvement was obtained or when autologous MSCs were no longer available (2–5 injections). Sustained complete closure of fistula tract was observed in 7/10 patients and incomplete closure in 3/10 patients, with images of regenerative tissue without fibrotic tissue obtained at MRI. A significant reduction of Crohn's disease activity index (CDAI) ($P < 0.001$) and PDAI ($P < 0.001$) was observed in all patients, with remission (CDAI ≤ 150, PDAI ≤ 8) obtained usually after the second procedure. Rectal mucosal healing, assessed by lower endoscopic examination at the end of the follow-up period, was demonstrated in 7/7 patients. Interestingly, there was a significant increase in the percentage of circulating $CD4^+CD25^{bright}FoxP3^+$ regulatory T cells (as early as after the second injection, and stable at 12 months) ($P < 0.01$) and mucosal $FoxP3^+$ regulatory T cells in the inflamed areas (at 12 months) ($P < 0.0001$). There was no adverse event during the procedure nor during the 12-month follow-up period.[100]

Allogeneic MSC therapy for fistulising Crohn's disease. In a single-arm, multicentre phase I/IIa clinical trial, 24 patients suffering from complex perianal fistula associated with non-active luminal Crohn's disease received an intrafistular treatment with 20 million allogeneic adipose tissue-derived MSCs, followed by a second administration of 40 million adipose tissue-derived MSCs if fistula closure was incomplete at week 12. Improvement of ≥1 fistulae was observed in 12/20 patients at week 12 and 9/13 at week 24, while complete closure (assessed by clinical examination) occurred in 8/21 patients at week 12 and 9/16 at week 24. There were statistically significant decreases of MRI Score of Severity (MSS) at week 12 and of PDAI (by more than 37% compared to baseline; $P < 0.01$), while no effect was observed on the CDAI. After a 6-month follow-up, the safety profile was acceptable with a low number of serious adverse events (including anal abscess in three patients and uterine leiomyoma in one patient).[101] Finally, the first results of a recent phase III, double-blind, randomised trial have been published: 212 patients with refractory complex active perianal fistulas associated with non-active luminal Crohn's disease were treated with surgical closure of the internal opening of the fistula(s) followed by a single injection of either 120 × 10^6 adipose tissue-derived MSCs or placebo into the tissue adjacent to all fistula tracts and internal openings, in addition to their current treatment. After 24 weeks, there was a significantly greater proportion of patients achieving fistula closure (assessed by both clinical and MRI evaluation) in the MSC group compared with placebo (53/107 vs. 36/105, $P = 0.024$, RR 1.42), with a

significantly shorter time to obtain clinical remission (closure of all treated external openings) in the MSC group (6.7 vs. 14.6 weeks). Compared with placebo, MSC therapy was associated with a significantly greater improvement in PDAI at week 6, but not at week 24. MSC injection was well tolerated (17.5% patients experiencing treatment-related adverse events vs. 29.4% in the placebo group, the most common being anal abscess and proctalgia).[102]

The efficacy of allogeneic bone marrow MSCs was also assessed in a phase IIa trial, in which 21 patients suffering from refractory perianal fistulising Crohn's disease were randomly assigned to groups given injections of 1×10^7, 3×10^7 or 9×10^7 MSCs, or placebo (solution with no cells), into the wall of the fistula, around the internal opening. Healing was observed in 8/15 patients in the MSC groups at week 6 and 7/15 patients at week 12 vs. 1/6 and 2/6 patients in the placebo group at the same time points. These effects were maintained until week 24. Overall, better results were obtained in the 1×10^7 and 3×10^7 MSC groups compared with the 9×10^7 MSC group.[103]

MSC therapy for luminal IBD

Intravenous injection of autologous MSCs has only been evaluated in two small trials, with underwhelming results, while intravenous injections of allogeneic MSCs have been more successfully performed in several clinical studies (Table 3). Thirty-one cases of refractory luminal Crohn's disease or ulcerative colitis treated with systemic infusions of allogeneic MSCs have been reported, and the results were promising, with around 60% of patients experiencing a response and around 40% achieving clinical remission, but MSCs still have to prove their superiority to placebo in this setting. Many clinical trials are also currently including patients suffering from Crohn's disease or ulcerative colitis (Table 4), using mostly allogeneic bone marrow, adipose tissue-derived or umbilical cord MSCs, but also, in one trial, autologous MSCs.

Autologous MSC therapy for luminal IBD. Intravenous injection of autologous bone marrow MSCs has been evaluated in two small phase I trials. In the first trial, nine patients with moderate-to-severe refractory Crohn's disease (CDAI between 220 and 450, refractory to medical treatments including anti-TNF therapy) received two intravenous injections of $1–2 \times 10^6$ cells/kg, 7 days apart. The authors observed a clinical response (decrease in CDAI by ≥70 points) in 3/9 patients but no clinical remission (CDAI <150) and even significant worsening of the disease in 4/9 patients, requiring surgery or rescue medication within 14 weeks after treatment, despite a trend for lower $CD4^+$ T-cell and higher $CD4^+CD127^+$ Treg numbers in the inflamed mucosa.[104] In the second trial, 12 patients with moderate-to-severe refractory Crohn's disease (CDAI >220, refractory to medical treatments including anti-TNF therapy) received one infusion of 1, 5 or 10×10^6 MSCs/kg. Clinical response (decrease in CDAI by ≥100 points) was observed in 5/11 patients 2 weeks after infusion (two patients in the 2×10^6 group, one in the 5×10^6 group and two in the 10×10^6 group), but 5/11 patients experienced a worsening of Crohn's disease during the 9-week follow-up (two patients in the 2×10^6 group, one in the 5×10^6 group and two in the 10×10^6 group). Two serious adverse events were considered possibly related to MSC therapy: severe Crohn's colitis and appendicitis 9 days after infusion in one patient, and *Clostridium difficile* colitis 30 days after infusion in another patient.[105]

Allogeneic MSC therapy for luminal IBD. In a phase II pilot study, nine patients with refractory moderate-to-severe Crohn's disease (CDAI ≥220, unsuccessfully treated with steroids and immunomodulators) received an intravenous injection of 2×10^6 or 8×10^6 bone marrow MSCs/kg. After 28 days, CDAI was decreased in all patients (mean decrease of 105, $P = 0.004$), with clinical response (reduction in CDAI ≥100) in 3/9 patients, among which one patient was even in clinical remission (CDAI <150). All patients reported an increased quality of life by day 28 ($P = 0.008$). No infusion reaction was observed, and five patients experienced mild to moderate adverse events.[106] Another group led a phase I clinical trial in which seven patients with IBD (four patients with Crohn's disease and three with ulcerative colitis) received one intravenous infusion of 1×10^6/kg allogeneic MSCs (bone marrow MSCs from healthy family members or umbilical cord MSCs), while continuing their treatment with steroids and/or immunosuppressors. After 3 months, a significant reduction in CDAI/CAI was observed in all patients, with remission achieved in 5/7 patients (2/4 with Crohn's disease and 3/3 with ulcerative colitis) and endoscopic improvement in 3/4 patients (2/2 with Crohn's disease and 1/2 with ulcerative colitis). Remission lasted for more than 2 years in two patients, while two other patients relapsed at 6 and 7 months. One patient with Crohn's disease had a significant reduction in fistula size and drainage. Histological analysis of biopsy specimens showed a reduction of the extent of the inflamed area and of the lymphocytic infiltration in the lamina propria. No serious adverse effect

Table 3 | Completed trials – intravenous infusion(s) of MSC for luminal Crohn's disease or ulcerative colitis

Origin	Phase	*N*	Schema	Evaluation	Response
Autologous MSC therapy					
Bone marrow	I	9	2 injections of 1–2 × 10^6 MSCs/kg, 7 days apart	Clinical evaluation at week 14, colonoscopy at week 6	(i) Clinical response in 3 patients, worsening of the disease in 4 patients (ii) Endoscopic improvement in 2 patients[104]
Bone marrow	I	12	1 injection of 2, 5 or 10 × 10^6 MSCs/kg	Clinical evaluation at week 2; 9-week follow-up	(i) Clinical response (decrease in CDAI >100) in 5 patients (2 patients in the 2 × 10^6 group, 1 in the 5 × 10^6 group and 2 in the 10 × 10^6 group) (ii) Worsening of Crohn's disease in 5 patients during follow-up (2 patients in the 2 × 10^6 group, 2 in the 5 × 10^6 group and 1 in the 10 × 10^6 group) (iii) Serious adverse events possibly related to MSC therapy in 2 patients (severe Crohn's colitis and appendicitis; and *Clostridium Difficile* colitis)[105]
Allogeneic MSC therapy					
Bone marrow	II	9	2 or 8 × 10^6 MSCs/kg	Clinical evaluation at day 28	(i) Decrease in CDAI in all patients, with clinical response in 3/9 patients and clinical remission in 1 patient (ii) Increased quality of life[106]
Bone marrow (familial) or umbilical cord	I	7*	1 × 10^6 MSCs/kg	Clinical evaluation at month 3; 6-month follow-up	(i) Significant reduction in CDAI/CAI in all patients, with remission in 5/7 patients (ii) Endoscopic improvement in 3/7 patients (iii) Reduction in fistula size and drainage in 1 patient (iv) Reduction in extent of the inflamed area and in lymphocytic infiltration in mucosa propria[107]
Bone marrow	II	15	4 weekly infusions of 2 × 10^6 MSCs/kg	Clinical, endoscopic and biological evaluation 42 days after the first injection	(i) Clinical response in 12/15 patients, clinical remission in 8/15 patients (ii) Endoscopic improvement in 7/15 patients (iii) Improved quality of life[108] (iv) Normalisation of CRP levels in 2/7 patients

* Four patients with Crohn's disease and three patients with ulcerative colitis.

was reported, after a mean follow-up of 19 months (range 6–32 months).[107] Finally, in a phase II study, 16 patients with refractory active luminal Crohn's disease (CDAI >250, refractory to infliximab or adalimumab) received weekly intravenous infusions of 2 × 10^6/kg allogeneic bone marrow MSCs for 4 weeks. After 42 days, the authors observed a reduction of the mean CDAI scores after each MSC infusion and improved mean quality of life scores. Clinical response occurred in 12/15 patients, clinical remission in 8/15 patients and endoscopic improvement in 7/15 patients. Normalisation of CRP levels was observed in 2/7 patients.[108]

Issues to be resolved

Sources and origins of MSCs. Most of the published human trials on fistulising Crohn's disease were performed with adipose tissue-derived MSCs[95–98, 101, 102]; only two studies used bone marrow MSCs.[100, 103] On the contrary, only bone marrow or umbilical cord MSCs have been used as systemic therapy to treat luminal CD.[104–108] The greatest advantage of adipose tissue-derived MSCs is their accessibility in large number and using minimally invasive procedures. More and more data are available on the differences between bone marrow, adipose tissue-derived and umbilical cord MSCs, including on their immunomodulatory properties. Several studies suggest that adipose tissue-derived and umbilical cord MSCs might be superior to bone marrow MSCs in suppressing immune responses *in vitro*.[109–113] Therefore, studies comparing their efficacy *in vivo* will have to be performed in order to answer this question.

Table 4 | Ongoing protocols – allogeneic MSC therapy for luminal Crohn's disease and/or ulcerative colitis

	Disease	Localisation	Phase	Origin	Schema	
NCT 01540292	Luminal Crohn's disease	University Hospital of Liege, Belgium	I–II	Bone marrow	2 injections of 1.5–2.0 $\times$ 10^6 MSCs/kg body weight, 4 weeks apart	Recruiting
NCT 00482092, 01233960, 00543374	Luminal Crohn's disease	Mesoblast International, Canada	III	Bone marrow	4 injections over 2 weeks, of either 600 $\times$ 10^6 or 1.2 $\times$ 10^9 MSCs, or placebo	Active, not recruiting
NCT 02000362	Luminal Crohn's disease	Kang Stem Biotech, South Korea	I/IIa	Umbilical cord	1 IV injection of either 0.5 or 1 $\times$ 10^8 MSCs	Unknown
NCT 01221428	Ulcerative colitis	Qingdao University, China	I–II	Umbilical cord	1 IV injection of 2 $\times$ 10^7 MSCs, followed 1 week later by 1 injection of 1 $\times$ 10^7 MSCs in the mesenteric artery	Unknown
NCT 01914887	Ulcerative colitis	Instituto de Investigación Hospital Universitario La Paz, Bolivia	I–II	Adipose tissue	Disseminated endoscopic injections of 60 $\times$ 10^6 cells in the affected colonic submucosa	Unknown
NCT 02442037	Ulcerative colitis	Affiliated Hospital to Academy of Military Medical Sciences, China	I-II	Umbilical cord	3 weekly IV infusions of either 1 $\times$ 10^6 MSCs/kg or placebo	Recruiting
NCT 02150551	Luminal Crohn's disease or ulcerative colitis in children	Children's National Medical Center, Washington, USA	I	Bone marrow	8 weekly IV infusions of 1 $\times$ 10^6 cells/kg	Recruiting

Both autologous and allogeneic MSCs have demonstrated some efficacy in fistulising Crohn's disease, while allogeneic MSCs have shown more promising results than autologous MSCs in luminal Crohn's disease. The use of autologous MSCs is debated since MSCs could be altered in patients with Crohn's disease. Indeed, in other auto-immune diseases, such as SLE, MSCs have lower proliferation rates,[114] and the use of autologous MSCs in SLE has led to disappointing results. However, when compared to MSCs from healthy donors, MSCs from patients with Crohn's disease have similar growth potential and T-cell suppression properties *in vitro*.[104, 115] Adipose tissue-derived MSCs from donors with Crohn's disease have also demonstrated their therapeutic effect on DSS-induced colitis in mice.[115] Nevertheless, allogeneic MSCs represent a more consistent source of cells and have the advantage that they can be used regardless of the centre's expertise generating autologous MSCs.

Dosage and modalities of administration. Concerning fistulising Crohn's disease, local injection of MSCs is indubitably the most interesting route of administration. In most recent trials,[102] the injection was performed in the fistula wall itself and was associated to a surgical closure of the inner opening of the fistula. These details of local injection may have an important impact on the observed results and should be further clarified. On the contrary, in luminal Crohn's disease, the extent and dissemination of lesions impose systemic administration. Both intravenous and intraperitoneal injections are efficient in mice, except in one study with cryopreserved MSCs, where only the intraperitoneal administration allowed migration to the inflamed colon and attenuation of colitis.[76] In patients, intravenous injection is easy, minimally invasive and safe. However, it is known that many MSCs are stopped in the lungs, and the proportion of MSCs reaching the inflamed intestine has not been evaluated. Infusion of MSCs via the mesenteric artery may increase the amount of MSCs reaching the inflamed organ and has been successfully performed in one patient without any complication.[116] However, this route of administration is clearly more invasive, and its interest appears minimal as intravenous infusion seems efficient.

Trials showing efficacy of intravenous MSCs have used different doses and schedules of administration, with a total dose varying from 1 to 10 million MSCs per kilo body weight. In two small studies, authors observed similar results in the different dose groups.[105, 106] Promising

results have been obtained whether patients received a single injection of MSCs[106, 107] or repeated injections,[108] but no long-term follow-up data are available. In fistulising Crohn's disease, the number of injected MSCs varied depending on the trial, but the injection schedule was more constant: most authors chose to realise a single injection of MSCs, sometimes repeated after 6–8 weeks (with a similar or higher dose) only if a complete closure of the fistula was not obtained, and in most cases, fistula improvement or healing was sustained up to 1 year.[95–98, 101, 103] Results were uniformly positive regardless of the number of MSCs; however, in a recent phase II trial, better results were obtained in the groups receiving 1×10^7 or 3×10^7 MSCs compared with the group receiving 9×10^7 MSCs.[103] Therefore, the optimal amount of MSCs and the best schedule of treatment are yet to be determined, and dose escalation trials will have to be performed to resolve this issue.

Ex vivo *strategies to modify MSCs.* As mentioned above, IFN-γ-primed-MSCs have demonstrated more potent immunosuppressive properties *in vitro* and were superior to treat murine colitis.[69, 70] These findings led to the assumption that the *in vivo* efficacy of resting MSCs relies on their activation by pro-inflammatory cytokines when administered after the onset of inflammation. This is corroborated by the demonstration that adipose tissue-derived MSCs increased survival rate, ameliorated body weight loss and improved colon inflammation in DSS-induced colitis when injected at day 2, but were ineffective when injected 1 day or 1 week before colitis induction.[36] Similar results have been obtained in models of other inflammatory conditions, such as graft-versus-host disease[117] and delayed-type hypersensitivity.[68] However, there is a recent case report of failure of IFN-γ pre-treated MSCs to treat a child with refractory Crohn's disease.[118]

Conventional culture methods involve the use of foetal bovine serum as a source of growth factors, but some concerns have been raised about infection transmission and immunogenicity. Indeed, foetal bovine serum proteins are internalised by MSCs, and anti-foetal bovine serum antibodies have been detected in the serum of patients treated with MSCs, which might trigger immunological reactions and lysis of MSCs *in vivo*.[119] Human platelet lysate is a human alternative that has gained clinical interest due to its properties to accelerate wound healing; importantly, human cord blood-derived platelet lysate co-injected with adipose tissue-derived MSCs isolated from Crohn's disease patients enhanced their therapeutic activity in a mouse model of DSS-induced colitis.[120] *In vitro*, human platelet lysate is superior to foetal bovine serum for stimulation of MSC proliferation (particularly adipose tissue-derived MSCs), without impairing their immunosuppressive properties, but effects on chemokine receptor and integrin expression have still to be clarified.[121, 122] In most experiments on murine colitis and human Crohn's disease, MSCs were cultivated with foetal bovine serum; there were only two trials with MSCs expanded with human platelet lysate, resulting in similar results than in studies using MSCs expanded with foetal bovine serum.[100, 105] Interestingly, in other conditions such as graft-versus-host disease (GVHD), recent meta-analyses found no difference in the outcome of patients treated with systemic infusions of MSCs cultivated with human platelet lysate or foetal bovine serum.[123, 124]

As mentioned above, MSC efficacy in tissue repair is conditioned by their ability to home into sites of inflammation, where they secrete humoral factors such as cytokines that improve the function of local cells. The fate of MSCs after local or systemic administration is still debated, and some data might be biased by the detection limits of the methods used. For instance, Devine *et al.* studied the engraftment of GFP-marked autologous or allogeneic MSCs in nonhuman primates following total body irradiation; in a first experiment, MSCs were detected (by semi-quantitative polymerase chain reaction) only in bone marrow biopsies in all five recipients,[125] while in a second experiment, they were able to detect (by reverse transcriptase polymerase chain reaction) a prolonged (9–21 months) engraftment in many organs and tissues.[72] In humans, MSCs do not seem to engraft durably, with MSC donor DNA detection decreasing in a time-dependent manner.[126] In order to enhance long-term beneficial effects of MSCs, attempts are being made to increase this engraftment. In several animal models of myocardial infarction, pulmonary fibrosis or diabetic erectile dysfunction, pre-conditioning of MSCs in hypoxic conditions or with antioxidants molecules (such as high-density lipoproteins, curcumin, atorvastatin, melatonin and several others) increased their expression of chemokines such as CXCR4 and their survival capacities, resulting in enhanced homing and engraftment, and ultimately in better efficacy.[127] These strategies should be studied and implemented in the setting of IBD (either systemic infusion of MSCs for luminal Crohn's disease or local injection for fistulising Crohn's disease) in order to ameliorate both overall and sustained response rates.

Concomitant use of other drugs. Mesenchymal stromal cells are used alongside with immunosuppressive drugs in clinical studies, and they share common targets. Their interaction should then be studied carefully. *In vitro*, exposure of MSCs to physiological concentrations of azathioprine, methotrexate, mercaptopurine and anti-TNFα agents does not affect their survival and their inhibitory capacities on peripheral blood mononuclear cell proliferation. There might even be an additive effect with mercaptopurine and anti-TNFα antibodies.[128] However, at higher *in vitro* concentrations, azathioprine can decrease the proliferation of rat bone marrow MSCs and increase their apoptosis and necrosis.[129] The influence of immunosuppressive drugs on IFN-γ secretion should also be more carefully studied, since activation of MSCs by IFN-γ seems to be important for their action. In murine cardiac allograft, infusion of allogeneic MSCs in combination with mycophenolate mofetil, which does not affect IFN-γ production by activated T cells, prolonged graft survival, while the combination of allogeneic MSCs with CsA, which completely abrogates IFN-γ production by activated T cells, reduced their efficacy.[130, 131]

Safety

No major MSC-related adverse event has been reported so far, whether in clinical trials with patients suffering from IBD, or in other diseases.[132–138] A few studies have shown mild and transient fever during or right after injection, and this association was significant in a meta-analysis.[138] No ectopic growth of tissues has been associated with local injections. Nevertheless, the apparent absence of long-term engraftment might protect against this risk, and a special attention should be paid to MSCs that have been pre-treated with the aim of enhancing their *in vivo* survival. Despite their immunosuppressive properties, MSCs did not increase the risks of infection or malignancy in clinical trials.[138] Some animal studies suggested that MSCs could promote tumour growth,[139, 140] or conversely have a tumour-suppressive activity.[141–143] These contradictory results could be explained by a context-dependent role of MSCs in regulating tumour growth.[144, 145] In a small pilot human trial in haematologic malignancies, co-transplantation of MSCs and haematopoietic stem cells was apparently associated with a higher relapse rate compared with control (60% vs. 20%), but this has never been confirmed by other studies.[146] In two studies using a murine model of colitis-associated tumorigenesis induced by azoxymethane and DSS, MSC therapy even resulted in a significant suppression of colitis-related neoplasm (reduction in tumour numbers compared to controls), probably because of a reduction of the inflammatory environment. A decreased expression of pro-inflammatory cytokines and a down-regulation of STAT3 phosphorylation have been implicated,[147] as well as an enhanced induction of Tregs from naive T cells by MSC-secreted TGF-β.[148] Clinical trials with long-term follow-up should be performed to definitively assess the safety of MSC therapy.

CONCLUSIONS

Mesenchymal stromal cell therapy represents a hope for the treatment of many dysimmune diseases. As they exert both immunomodulatory effects and tissue repair promotion, MSCs seem particularly promising in IBD, where a dysregulated immune system causes tissue damages. Currently, the results of clinical trials are particularly encouraging in perianal fistulising Crohn's disease. Indeed, MSCs have demonstrated their ability to heal perianal Crohn's disease fistulae in patients refractory to conventional or biologic therapy in several controlled trials. Moreover, for fistulising perianal Crohn's disease, MSC therapy is a minimally invasive procedure that, unlike current surgical options, does not injure the anal sphincter (and can even promote a nonfibrotic healing of fistulas). Unlike conventional immunosuppressive drugs, it does not seem to increase the risk of opportunist infections. MSCs still have to demonstrate their efficacy in luminal Crohn's disease and in ulcerative colitis. To address this issue, phase II and III studies using both clinical remission and endoscopic response as co-primary endpoints should be performed. Furthermore, the optimal origin and sources of MSCs, as well as dosage and modalities of administration, have still to be determined. Future trials should aim to resolve these questions in order to optimally use the great potential of MSCs to treat IBD.

AUTHORSHIP

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