DOI: 10.4274/tjcd.galenos.2021.2021-10-6
Turk J Colorectal Dis 2022;32:161-169

REVIEW

Mesenchymal Stem Cells for Perianal Crohn's Disease

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ABSTRACT

Unfortunately, perianal fistulizing Crohn's disease (CD) is notoriously difficult to cure. The ulceration and inflammation in CD which leads to fistulizing disease is the likely reason fistulas are notoriously difficult to treat. Most studies which evaluated the efficacy of mesenchymal stem cells (MSCs) in perianal CD had small sample sizes, which warranted wider clinical trials. Some of the available data were case reports, small case series or single arm small studies. The largest pivotal trial published to date which evaluated efficacy and safety of MSCs in perianal fistulas in CD was entitled the Adipose Derived Mesenchymal Stem Cells for Induction of Remission in Perianal Fistulizing Crohn's disease trial. MCS administration retains a high potential value in the treatment of perianal CD.

Keywords: Perianal fistula, mesenchymal stem cell, Crohn's diseas

Introduction

Crohn's disease (CD) is a chronic inflammatory disease of the gastrointestinal tract of unknown etiology, which continues to increase in incidence for unknown reasons, resulting in a significant burden to the healthcare system.[1,2] CD is characterized by persistent transmural inflammation anywhere along the gastrointestinal tract with a chronic remitting and relapsing behavior, which leaves patients on chronic immunosuppression and recurrent operations to treat the disease symptoms, but neither are curative for the disease. Perianal CD, present in over 25% of patients with CD, is notoriously difficult to treat with currently available biologics and surgical procedures. These patients experience significant morbidity due to pain, persistent drainage, recurrent perianal sepsis, and ongoing need to access medical care, resulting in increased costs[21] and impaired quality of life.[2]

Unfortunately, perianal fistulizing CD is extremely difficult to cure with 37% of patients experiencing refractory disease.[3] As a result, patients cycle through numerous immunosuppressive medications that can have significant side effects, and more than 90% undergo multiple surgical interventions[4] putting them at risk of incontinence.[5] While up to 64% can achieve fistula healing with optimized tissue flaps[5] the majority of patients cannot have a flap constructed, and 40% of patients are left with active disease, facing a lifetime of debilitating morbidity or, alternatively, a proctectomy.[6,7]

The current ineffective treatment paradigm leaves patients with incontinence, chronic narcotics, lost jobs, increased risk of opportunistic infection from biologics and increased incontinence from surgical intervention, and significantly impaired quality of life in thousands of patients. This dismal picture has spurred significant interest in investigating better treatment options that have the potential for improved efficacy without a risk of incontinence.

Mesenchymal Stem Cells for Perianal Fistulas

The ulceration and inflammation in CD that leads to fistulizing disease is the likely reason fistulas are notoriously difficult to treat.[8] The successful use of mesenchymal stem cells (MSCs) for the treatment of a refractory rectovaginal fistula in the setting of CD was first reported in 2003.[13] These promising results generated a wave of phase I,[14-19] phase II[14,20,21] and phase III[20] trials to study the safety and efficacy of using MSCs to treat perianal CD. Despite the heterogeneity in protocols using allogeneic[14,16,19,20] or autologous MSCs[13-15,17,18,21,22] derived from both bone marrow[19,22] or adipose tissue,[13,16-18,20] administered at various doses, delivered as a singular or repeated injection, and delivered with[16,17,20] or without scaffolding,[19,23] the results of all completed trials have been encouraging with regard to both safety and efficacy (Table 1).

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Received: 25.10.2021 **Accepted:** 27.12.2021

Table 1. Summary of prior studies of mesenchymal stem cells for perianal Crohn's disease

Name of study	Type of study	Location	Patients with CD#	Intervention
García-Olmo et al.[13]	Case report	Spain	1	Local injection of stem cells
García-Olmo et al.[17]	Phase I, open label, single arm	Spain	4	Local injection of $3x10^6$ million MSC
García-Olmo et al.[18]	Phase IIb, open label, double arm, randomized	Spain	14	Local injection of $2x10^6$ MSC plus fibrin glue as compared to fibrin glue alone; second dose of $4x10^6$ MSC if fistula healing was not seen at 8 weeks
Cho et al.[15]	Phase I, open label, single arm	Korea	10	$1x10^7$,$2x10^7$,$4x10^7$, cells/mL based on the size of the fistula (total of 3-40$x10^7$ cells)
Lee et al.[14]	Phase II, open label, single arm	Korea	33	$3x10^7$ or $6x10^7$ cells per 1 cm of fistula length; average number of $15.8x10^7$ cells), followed by a second injection of 1.5 times more cells (average number of $19.1x10^7$ cells) if fistula closure was not complete at 8 weeks
Cho et al.[21]	Phase II extension of Lee phase II	Korea	24	9-42$x10^7$ cells based on length of fistula tract
Ciccocioppo et al.[22]	Open label, single arm	Italy	10	1.5 to $3x10^7$ MSC every 4 weeks until an improvement was obtained or when autologous MSCs were no longer available (2-5 injections)
de la Portilla et al.	Phase I/IIa open label, single arm	Spain	24	Local injection of $2x10^6$ MSCs; second injection of $4x10^6$ if unhealed at 14 weeks
Panes et al.[20]	Phase III, RCT	Europe/Israel	212	Local injection of stem cells
Molenkijk et al.[19]	Open label, 4 arms	Netherlands	21	n=5 in 10^7 MSC dose (G1) n=5 in $3x10^7$ MSC dose (G2) n=5 in $9x10^7$ MSC dose (G3) n=6 in placebo (G4)
Dietz et al.[16]	Phase I, open label, single arm	USA	12	20 million cells on a GORE Bio A Plug
Panes et al.[23]	Phase III, RCT	Europe/Israel	212	Local injection of stem cells
Barnhoon	Phase I	Europe	15	Local injection of stem cells

RCT: Randomized controlled trial, MSC: Mesenchymal stem cell, MRI: Magnetic resonance imaging, SAE: Serious adverse event, AE: adverse event, TEAEs: Treatment-emergent adverse event, CD: Crohn's disease

Type and source of stem cells	Outcome	Results	Use of MRI	Adverse events
Autologous, adipose tissue	Complete epithelialization of external opening	Fistula healed in 1 week, no recurrence till 3 months post treatment	No	None
Autologous, adipose tissue	Complete epithelialization of external opening	3 of 4 rectovaginal or perianal fistula (75%) at 8 weeks	No	None
Autologous, adipose tissue	Complete epithelialization of external opening	5 of 7 fistulas (71%) in MSC versus 1 of 7 fistulas (14%) healed in fibrin glue alone at 8 weeks	No	15 non-serious AE; 4 serious AE, 1 related to MSCs (perinala abscess)
Autologous, adipose tissue	Complete epithelialization of external opening	3 of 10 patients (30%) had complete healing at 8 weeks post treatment; sustained at 8 months	No	13 AE were reported in seven patients (70%); 3 SAE in 2 patients (20%, one related with seton placement
Autologous, adipose tissue	Complete epithelialization of external opening	27 of 33 patients (82%) had complete healing at 8 weeks; 88% sustained closure at one year	No	28 AE, all unrelated to MSC; 1 SAE unrelated to MSC
Autologous, adipose tissue	Complete epithelialization of external opening	20 of 24 patients (83%) had sustained closure at two years	No	53 AE, all unrelated to MSC
Autologous, adipose	No drainage on clinical exam as well as healed on MRI	6 of 9 patients (67%) with complete closure at 8 weeks; all sustained closure at one year	Yes	No adverse events
Allogeneic, adipose tissue	absence of drainage and complete epithelization, 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	Four SAE (three anal abscesses and one uterine leimyoma), so the group concluded the treatment had an acceptable safety profile
Allogeneic, adipose tissue	Absence of drainage and <2 cm fluid collection on MRI	50% (n=53 of 107) healed in the MSC group compared with 34% (n=36 of 105, p=0.024) at 24 weeks	Yes	Overall, 68 (66%) in treatment, 66 in placebo (65%); SAE in 18 (17%) and 14 (14%), majority anal abscess
Allogeneic, adipose	Absence of drainage and <2 cm fluid collection	12-week fistula healing: G1: 2/5 G2: 4/5 G3: 1/5 G4:2/6	Yes	50 AE, most common was common cold, 4 abscesses
Autologous adipose tissue on matrix	Absence of drainage and improvement in Van Assche score on MRI	10 of 12 patients with healing at 6 months (83%)	Yes	No adverse events
Allogeneic, adipose tissue	Absence of drainage and <2 cm fluid collection on MRI	57% (n=49 of 86) healed in the MSC group compared with 39% (n=33 of 84, p=0.021) at 52 weeks	Yes	Most common anal pain/ abscess, study withdraw <10% related to TEAEs
Allogeneic, bone marrow tissue	Absence of drainage and <2 cm fluid collection on MRI	13/15 (87%) available for 4-year f/u. healing maintained from 1-year results	Yes	No increased adverse events from 1-year results

Mechanism of Action of Mesenchymal Stem Cells

While the exact mechanism of MSCs in treating CD remains unknown, it is well established that MSCs exist in almost all tissues[24-26] and are believed to reduce exacerbated inflammation due to their intrinsic immunomodulatory properties. Recently, success of MSCs in treating severe inflammatory disorders, such as graft-versus-host disease[27,28] systemic lupus erythematosus,[29] myocardial infarction,[30] multiple sclerosis[31] and CD,[17] has highlighted the therapeutic benefit of the immunomodulatory characteristics of MSCs.[32-34] These immunomodulatory properties are carried out through three important steps: 1) migration to sites of active inflammation or tissue injury;[35-37] 2) secretion of anti-inflammatory molecules, such as interleukin-10, hepatocyte growth factor, transforming growth factor-beta-1[38], and indoleamine 2,3-dioxygenase;[39] and 3) paracrine signaling to nearby cells to maintain the local anti-inflammatory environment (Figure 1).[40,41] By influencing cytokine secretion profiles,[42] MSCs can modulate the function of various immune cell types including lymphocytes, dendritic cells and macrophages.[43] Significant and specific for CD is the ability of MSCs to upregulate a $CD4^+$ T-cell subset of regulatory T-cells (Tregs), a cell type known to be deficient in CD.[25,44] It has been well established that the depletion of Tregs and imbalance of Tregs with T-effector cells plays a key role in the pathogenesis of CD.[45,46] Therefore, the ability of MSCs to upregulate Tregs, migrate to sites of inflammation,[47] and dampen immune responses underscores the escalating interest in using MSCs to treat CD.[48-52]

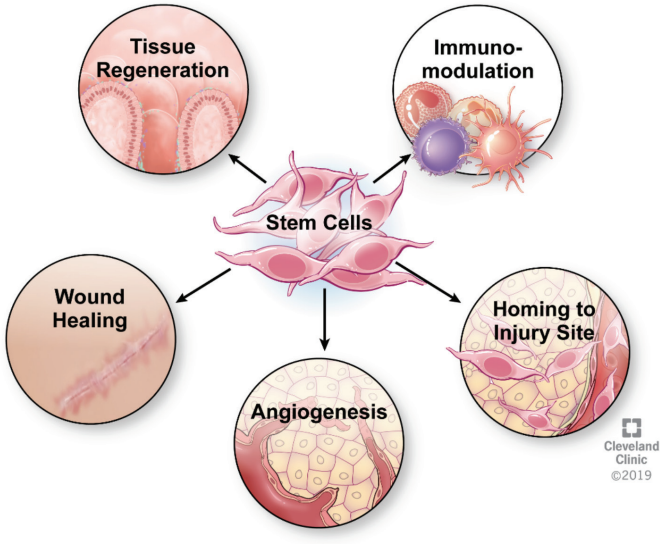

Figure 1. Mechanism of action of MCS
MSC: Mesenchymal stem cell

Application and Results of MSC in Perianal Fistulizing Crohn's Disease

Indications for the use of MSCs in perianal CD are mostly confined to fistulas. This is described in the label of the commercially approved product available in Europe (Alofiselä, Darvadstrocel, Takeda Pharma A/S, Taastrup, Denmark). According to the label, the product is indicated for treatment of complex perianal fistulas in adult patients with non-active/mildly active luminal CD, when fistulas have shown an inadequate response to at least one conventional or biologic therapy.[53,64] The product needs to be used after surgical conditioning of the fistula, with curettage of the track and closure of the internal opening with a stitch. Despite this, there is a rationale for injection of MSCs in other situations. After commercial approval, indications for the use of stem cells in perianal CD in other phenotypes will probably be explored further, for example in rectovaginal fistulas or persistent ulcers.[19]

Most studies, which have evaluated the efficacy of MSCs in perianal CD, had small sample sizes, which warranted wider clinical trials. Some of the available data were case reports, small case series or single arm small studies. The largest pivotal trial published to date which evaluated efficacy and safety of MSCs in perianal fistulas in CD was entitled the Adipose Derived Mesenchymal Stem Cells for Induction of Remission in Perianal Fistulizing Crohn's Disease (ADMIRE-CD) trial.[20] The trial was a randomized, double-blind, placebo-controlled study that tested Cx601, a 24 mL solution with 120 million expanded adipose-derived MSCs in CD fistulas. Each vial of the product had 30 million cells, and a total of four vials of the product was used in each case. The main inclusion criterion was patients with inactive or mildly active luminal CD (CDAI of 220 or less) with associated complex perianal fistulas. Patients with active proctitis, rectal stenosis, ileostomies, colostomies and rectovaginal fistulas were excluded.

All patients had a previous surgical procedure under anesthesia, with curettage of the fistula tract(s) and seton placement, if needed (two weeks before the injection of the drug). In the main surgical procedure, an unblinded surgeon injected the MSC preparation or placebo saline solution (randomized in a 1:1 ratio) in the internal opening and close to the fistula tracts, after simple closure of the internal opening with stitches. The surgeon had to be unblinded as there were evident differences between the compound and saline solution in the pre-filled syringes.

The main objective of the study was to analyze combined remission (clinical closure of all treated external openings draining initially at baseline, and the absence of collections with more than 2 cm, confirmed by [magnetic resonance

imaging (MRI)] after 24 weeks, performed by blinded gastroenterologists and radiologists.

A total of 107 patients had Darvadstrocel injections and 105 had saline injections, as a control group. After 24 weeks, more patients in the Darvadstrocel group exhibited combined remission as compared to controls [53/107 (50%) versus 36/105 (34%), respectively; with a delta of 15.2% and 97.5% confidence interval 0.2-30.3; p=0.024]. Clinical remission alone (closure of 100% of external openings) was observed in 57% of the Darvadstrocel/Cx601 patients as compared to 41% of placebo (p=0.064). Clinical response was another secondary endpoint (closure of 50% of the fistula openings) and it was observed in 71% of the Darvadstrocel group as compared to 53% of placebo patients (p=0.054). Results are illustrated in Figure 2. In terms of safety, a total of 66% (68/103) of patients in the Darvadstrocel group and 65% (66/102) in control group had post-treatment adverse events, with proctalgia, anal abscess and nasopharyngitis being the most common. Treatment-related adverse effects were found in 17% in the study group as compared to 29% in placebo, mostly anal abscesses and proctalgia. Perianal abscesses occurred in 5% of the overall patients in both groups.

The long-term results (outcomes after 52 weeks) of the same trial were published in 2018.[23] The patients from the ADMIRE-CD study were followed up to 52 weeks and an additional MRI and a clinical evaluation were performed to check the same endpoints. Combined clinical and radiological remission was observed in 58/103 (56.3%) of the Darvadstrocel/Cx601 patients, as compared to 39/101 (38.6%) in the control group, with a delta of 17.7 points, 95% confidence interval: 4.2-31.2; p=0.010). Clinical remission (100% closure of baseline fistulas) after one year was observed in 59.2% in Darvadstrocel/Cx601 and 41.6% in placebo groups, respectively (p=0.013). Clinical response was observed in 66% and 55.4% in both groups, respectively (p=0.128). These findings are illustrated in Figure 3. Importantly, from the safety perspective, anal abscesses and fistulas were observed similarly between the groups in the 1-year analysis (33% of the active group and 29.4% in the placebo group). Serious abscesses/fistulas were observed in only 6.8% and 4.9% in both groups, respectively. The rates of withdrawal from the study due to adverse events were low between the groups, 8.7% and 8.8% respectively. No new safety signal in terms of new adverse events was observed in the additional 24 weeks of this long-term study.

A similar study is currently ongoing in the United States (Adult Allogeneic Expanded Adipose-Derived Stem Cells (eASC) for the Treatment of Complex Perianal Fistula(s) in Patients with Crohn's Disease-ADMIRE-CD-II) to demonstrate efficacy for a future approval of Darvadstrocel in America by the FDA (ADMIRE-CD-II trial, details available in clinicaltrials.gov). In Europe, a post-marketing registry entitled INSPIRE (design and implementation aspects of a registry of complex perianal fistulas in CD patients treated with Darvadstrocel) aims to establish a framework to capture real-world efficacy and safety data with this commercially available MSC product.[63] The registry is beginning to capture patients from different countries, and soon a more robust picture of patients who have undergone MSC local therapy will be available.

Safety

The risk of infection and tumor is of major concern with the use of MSCs. Indeed, the safety issue has yet to be fully addressed before the treatment is officially approved for its use on CD. While toxicity remains the most important limit for hematopoietic stem cell therapy in CD patients, MSCs have shown a relatively higher safety profile.[54] Serious adverse events (SAE) requiring hospital admission are rare and are more probably related to intrinsic disease

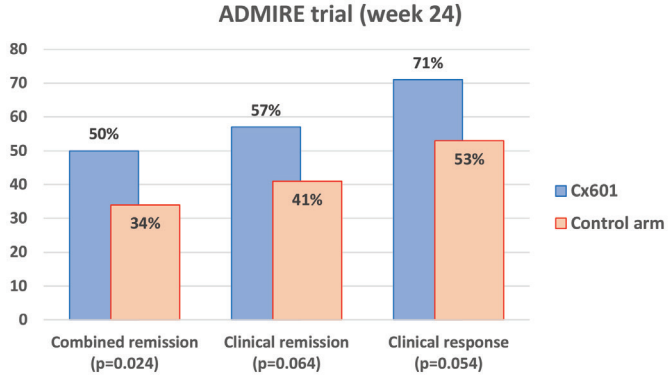

Figure 2. ADMIRE randomized trial results of efficacy at week 24

ADMIRE: Adipose Derived Mesenchymal Stem Cells for Induction of Remission in Perianal Fistulizing

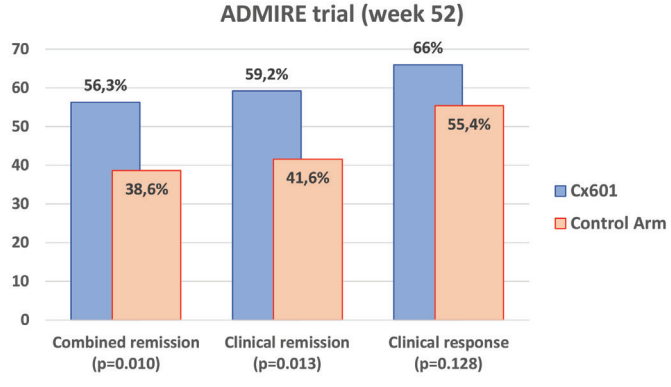

Figure 3. Long-term extension efficacy results of the ADMIRE randomized trial at week 52

ADMIRE: Adipose Derived Mesenchymal Stem Cells for Induction of Remission in Perianal Fistulizing

activity. The studies that have been published to date indicate that administration of MSCs might prompt minor adverse events, such as perianal sepsis. Indeed, a relatively high rate of perianal sepsis has been reported by phase I-II trials.[14,17,18] In the latest phase III trial published by Panés et al.[20], 68 patients (66%) in the treatment group and 66 (65%) in the control group developed AEs (adverse events), while SAEs were registered in 18 (17%) and in 14 (14%), respectively, the majority being anal abscess and proctalgia. In this study the rate of AEs and SAEs were comparable to the control groups. Arguably, the side effects have been interpreted as not directly related to MSC administration but rather to the procedure adopted for the fistula closure or preconditioning before MSC administration. Indeed a recent meta-analysis of comparative studies has shown no significant difference in AEs and SAEs when comparing MCS and non-MSC groups of patients.[55]

MSCs may show pro-tumorigenic impact in cancers, by inducing neoplastic cell proliferation and promoting angiogenesis.[56,57] To date, there are no reported cases of neoplasm developing after MCS perianal treatment. However, long-term follow up will clarify and strengthen this safety aspect.

Practical Considerations When Administering Stem Cell Therapy

Step 1 - Antibiotic Prophylaxis and Treatment

Currently, the knowledge of the potential effects of antibiotics on MSCs viability and function is scarce. However, some *in vitro* and animal studies suggest the most frequently used antibiotics (benzyl-penicillin, flucloxacillin, cefuroxime and metronidazole) have not shown any detrimental effects on the stem cells, while gentamicin and vancomycin may downregulate the proliferation and differentiation activity of MSCs.[1,2] Interestingly, bone marrow MSCs are reported to be able to take up ciprofloxacin and release it to the tissues, which could further increase the antibacterial effect of the stem cell therapy.[3,4] Until new data becomes available, we recommend standard antibiotic prophylaxis prior to surgery. In case antibacterial treatment is necessary after cells are implanted, we recommend avoiding gentamicin and vancomycin, if other alternatives are available.

Step 2 - Anesthesia

Any anesthesia protocol may be chosen, taking into consideration that the surgical insult is minimized with this technique. However, local anesthesia should be used with caution, due to the possible direct cytotoxic effect of the most frequently used anaesthetics (amide-type: ropivacaine, lidocaine, bupivacaine, and mepivacaine) to the MSCs, described after *in vitro* exposure of the cells to each of the drugs.[5] Furthermore, it was found that local anesthesia could directly and indirectly affect the anti-inflammatory capacity of MSCs, by altering the microenvironment, and modulating macrophage inflammation and MSCs secretion.[6] As local anesthesia in anal surgery is rarely applied, and in most cases, in the form of a pudendal block, the contact of the injected cells with the local anesthetics is not expected to occur and thus the surgical protocol may not be changed substantially. Nevertheless, if not strictly necessary, we recommend local anesthesia should be avoided.

STEP 3 - Surgical Preparation

Alcoholic, hydrogen peroxide and povidone-iodine solutions should be avoided in surgical preparation due to their toxicity to the cells. Polyhexamethylene biguanide, octenidine dihydrochloride and chlorhexidine (non-alcoholic) solutions seem to have the optimal profile for this purpose.[7,8] We tend to simply use normal saline with baby shampoo so that the preparation will not interfere with cell viability.

STEP 4 - Internal Fistula Orifice Location

Internal orifice location and management are the keys to successful treatment of perianal fistulas. Surgeons often inject hydrogen peroxide solution through the external opening to identify the internal opening. However, when stem cells are to be applied, in order to avoid the cytotoxic effects of the hydrogen peroxide, other methods should be employed. Probes or pure saline solution are appropriate for this purpose.

STEP 5 - De-Epithelization of the Fistula Tract

Extensive debridement of the epithelization creates an appropriate wound bed for the cells by exposing healthy tissue. We perform a deep mechanical debridement (curettage), especially of the internal orifice. Curettage is the single most effective and recognized part of fistula treatment. Bleeding from the external and internal opening should be observed to assure adequate debridement.

STEP 6 - Cleaning of the Cavities and Fistula Tracts

The tracts are cleaned with saline solution in order to remove devitalized tissue debris following curettage.

STEP 7 - Closure of the Internal Opening

We believe this surgical act should not be very aggressive. The closure should be achieved by simple 2/0 absorbable suture. The stitch must include full thickness bites, and snug pressure. Smaller and tighter bites may tear the fibrotic tissue.

STEP 8 - Stem Cell Handling and Resuspension

Stem cell handling is critical. This is a biological, living drug that comes to the operating theatre in the form of several

transparent (usually glass) vials and can be stored for a very limited time (24 hours after reception). Usually, the concentration used is 5-10 million cells/mL. Vials of cells are transported at regulated temperatures and are viable for fixed periods of time. Cells should be gently re-suspended by soft swinging movements, with care to avoid vigorous shaking. MSCs are characterized by their capacity to adhere to plastic surfaces. They should be aspirated with a large bore needle, such as a 16G.

STEP 9 - Stem Cell Injection

We recommend a slow injection process (to avoid high cell friction and cell mortality) through a fine and long needle (e.g., Abocatt 22G; Terumo). Studies have shown that up to 26G bore size needles are suitable for injecting MSCs without changing the viability and functional capacity of the cells, even after three passes through the needle.[9] We recommend injecting at least half of the total dose in the tissues around the internal orifice or orifices. The other half should be injected through the external orifice into the fistula walls in parallel to the tract.

Future perspectives of Stem Cell Therapy for Fistulas

Several unmet needs in the treatment of perianal CD with MSCs remains to be addressed. The most important issue is the presence of active proctitis during MSC administration. Perianal CD with associated variable grades of proctitis represents a relevant percentage of patients[58,59] that have been codified in the exclusion criteria of most trials. Indeed, one of the main issue in MSC administration remains to determine whether this treatment would be effective in the setting of active proctitis. Moreover, even though rarer, rectovaginal and enterocutaneous fistula patients have been excluded from the trials to date, and have limited treatment options. Thus, patients with these phenotypes may greatly benefit from MSC therapy.

The other crucial controversies regard the ideal cell dosage administration and the appropriate cellular delivery approach. In fact, no single cell dosage and administration procedure (direct injection, fibrin glue) has been consistently identified to date.[60] Once MSC administration becomes more mainstream, more widely available and, hopefully, cheaper preparation processes, and head-to-head comparison with standard therapy (including biologics and alternative surgical procedures) should be undertaken to validate the efficacy of this therapeutic approach. Furthermore, in order to overcome the issues noted and enhance the potential value of this treatment, the underlying mechanism with which MSCs promote tissue healing at the level of the fistula should be elucidated. Finally, studies addressing the impact of periodic MSCs administration are advocated to establish it as a maintenance therapy.

Conclusion

The management of perianal CD is controversial and currently used treatments have shown a relatively limited rate of success.[61] MSC administration retains a high potential value in the treatment of perianal CD. However, to date the procedure is considered as an alternative to standard medical therapy and supplementary surgical procedures.[62] Nonetheless, MSC administration is reported to be effective in inducing fistula healing but the mechanism promoting this healing is yet to be fully explored. Further studies are urgently required to determine the impact of MSC administration, and should also include complex fistulas with multiple fistula tracts, even in the presence of distal luminal disease. Of note, the lack of a widely accepted definition of fistula healing was problematic when we were comparing results of trials. Thus, a consensus definition of fistula healing should be created to further research into this promising therapeutic option for patients with perianal CD.

Peer-review: Internally peer-reviewed.

Financial Disclosure: The authors declared that this study received no financial support.

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