Journal of Crohn's and Colitis, 2023, **17**, 728–737
https://doi.org/10.1093/ecco-jcc/jjac192
Advance access publication 3 February 2023
Original Article

Clinical and MRI Evolution After Local Injection of Bone Marrow-Derived Mesenchymal Stem Cells in Perianal Fistulae in Crohn's Disease: Results From a Prospective Monocentric Study

Catherine Reenaers,[a] Romain P. Gillard,[b] Carla Coimbra,[c] René M. Gillard,[b] Paul Meunier,[b] Chantal Lechanteur,[d] Etienne Baudoux,[d] Layla Boutaffala,[a] Yves Beguin,[d,e] Édouard Louis[a]

[a]Gastroenterology Department, CHU Sart-Tilman, Liège, Belgium.
[b]Radiology Department, CHU Sart-Tilman, Liège, Belgium
[c]Abdominal Surgery Department, CHU Sart-Tilman, Liège, Belgium
[d]University of Liège, Laboratory of Cell and Gene Therapy LTCG, Liège, Belgium
[e]Hematology Department, CHU Sart-Tilman, Liège, Belgium

Corresponding author: Catherine Reenaers, MD, PhD, Gastroenterology Department, CHU Sart-Tilman, University of Liège, Avenue de l'Hôpital B37, 4000 Liège, Belgium. Tel: + 32 4 366 72 56; Email: catherine.reenaers@chuliege.be

Abstract

Background: Local injection of adipose tissue-derived mesenchymal stem cells [MSCs] is effective in fistulizing perianal Crohn's disease [CD]. Less is known about bone marrow-derived MSCs and little is known about predictive factors of response and magnetic resonance imaging [MRI] evolution of the fistulae after MSC injection. Our aims were to evaluate the safety and clinical outcome of bone marrow-derived MSC injection for perianal fistulizing CD, to evaluate the MRI evolution of the fistulae and to identify factors associated with fistula closure.

Patients and methods: All CD patients with perianal fistula and appropriate drainage with a seton without abscess at MRI were eligible. Clinical examination, biomarkers and pelvic MRI were performed at weeks 0, 12 and 48. The clinical outcome was assessed by closure of the treated external openings at clinical examination and MRI exploration.

Results: Sixteen patients with a median age of 49 years and a median duration of perianal CD of 8 months were included. No unexpected safety event occurred. At weeks 12 and 48, 9/16 and 8/16 patients had complete fistula[e] closure, respectively, whereas 11/16 patients had at least partial closure. At MRI, the degree of fibrosis increased significantly after MSC injection. In total, 86% of patients with >80% of fibrosis of the fistula tract at week 48 had fistula closure. Fistula closure at week 12 was predictive of fistula closure at week 48. The MAGNIFI-CD did not change significantly over time.

Conclusion: Open-label injection of bone marrow-derived MSCs was safe and was effective in half of the patients in fistulizing perianal CD and induced significant MRI changes associated with favourable clinical outcome.

Key Words: Perianal fistula; Crohn's disease; magnetic resonance imaging; fibrotic fistula tract

1. Introduction

Fistulizing perianal Crohn's disease [CD] is a disabling condition affecting up to 30% of patients during the course of the disease. It is associated with a low quality of life due to pain, discharge and formation of abscesses.[1–3] While the management of luminal CD has evolved in the last few years,[4] perianal fistulizing CD remains a major clinical challenge as the fistulae are often refractory to conventional medical and surgical treatments. After abscess drainage and temporary seton placement, medical or surgical techniques can be considered to close the fistula.[5–8] Since the development of infliximab [IFX], which was associated with 34% fistula closure at 46 weeks,[9] no new molecule has demonstrated a clear efficacy to close perianal fistulae in CD. Recently, Panes *et al.* reported allogenic, expanded, adipose-derived stem cells [Cx601] as a promising new therapeutic approach in fistulizing perianal CD with 51 and 56% fistula closure without abscess at pelvic magnetic resonance imaging [MRI] at weeks 26 and 52 respectively.[10] Fistula closure was also demonstrated with local injection of allogenic bone marrow-derived mesenchymal stromal cells [bmMSCs] derived from aspirates of healthy donors in a small placebo-controlled Dutch trial,[11] suggesting that not only adipose-derived but also bone marrow-derived MSCs could represent an effective strategy in complex fistulizing perianal CD, although this is currently hampered by the cost and logistics of administration of centrally produced MSCs. Hence, Cx601 treatment has not been made broadly accessible.

While the treat-to-target approach has been recently implemented in the management of luminal CD, including well-defined objectives and validated tools to regularly assess the patients,[4] no clear target or validated monitoring has been proposed yet in fistulizing perianal CD. To our knowledge,

no study has described the MRI evolution of perianal fistula after MSC injection over time. Although validated to describe fistulizing perianal CD, the Van Assche index[12] and more recently the MAGNIF-CD index[13] are not used in clinical practice as specific radiological targets for the follow-up of fistulae after medical or surgical treatment. Recently, van Rijn *et al*.[14] demonstrated that a low MAGNIFI CD index and high percentage of fibrosis of the fistula tract were associated with longer term fistula closure after anti-tumour necrosis factor [anti-TNF] or surgical treatment. The first aim of our study was to evaluate the safety and the clinical outcome after the injection of bmMSCs to treat perianal fistulizing CD. The secondary aims were to describe the MRI evolution of the fistula tract after MSC injection, to identify MRI and clinical factors associated with fistula closure, and to describe the impact on the quality of life of the patients.

2. Patients and Methods

2.1. Patient selection and study design

A prospective interventional uncontrolled study was performed in CHU Sart-Tilman, Liège, from October 2019 to October 2021. Eligible patients were adults [>17 years old] with a diagnosis of CD for at least 6 months and an active drained perianal fistula. Patients were excluded if they had an abscess [collection >2 cm], no appropriate seton drainage before inclusion, anal and/or rectal stricture, pregnant women or planning pregnancy within 1 year, positive stool culture/toxin for *Clostridium difficile* or other pathogens, documented human immunodeficiency virus infection, active hepatitis B, C or tuberculosis, an opportunistic infection within 6 months before screening or a serious infection in the previous 3 months, malignancy within the past 5 years, a history of lymphoproliferative disease, renal or hepatic failure, and patients unable to undergo MRI. All patients completed written informed consent before study inclusion.

The screening visit included clinical examination, evaluation of Crohn's disease Activity Index [CDAI], Patient Reported Outcomes [PROs] of stool frequency and abdominal pain, Perianal Disease Activity Index [PDAI], Short Health Scale [SHS], blood sampling for routine blood tests, and stool sampling for faecal calprotectin measurement. Pelvic MRI and sigmoidoscopy were performed within 2 months before inclusion. CD medications should be stable during the period of 12 weeks after bmMSC injection [type, dosage and frequency]. A rescue therapy with transient treatment intensification was allowed in case of clinical relapse. After MSC injection patients were evaluated at weeks 4, 12, 24, 36 and 48 with a systematic examination of the fistulous track and assessment of drainage or abscess formation, research of adverse events, questionnaire evaluation [CDAI, PROs, SHS], blood tests and faecal calprotectin measurement. Pelvic MRI was performed at weeks 12 and 48 during follow-up. PDAI was reassessed at week 48.

2.2. Preparation of mesenchymal stromal cells

Bone marrow collection and MSC expansion cultures were carried out at the Laboratory of Cell and Gene Therapy [LTCG] at the University of Liège as described in the manufacturer's standard operating procedure [TG0601PRO].[15,16] Briefly, bone marrow [50 mL] was collected under local anaesthesia in sterile conditions and placed in sterile heparin-containing tubes. Mononuclear bone marrow cells were isolated by an automated Ficoll procedure on the Sepax device, seeded in sterile tissue culture flasks and cultured in Dulbecco's modified Eagle's medium-low glucose with Glutamax supplemented with 10% gamma-irradiated fetal bovine serum. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2 for ~4 weeks. The medium was replaced twice a week and, after ~2 weeks, the cultures should be near confluence [>70%]. The cells were then detached by treatment with TryplE and re-plated [passaged] at a lower density to allow further expansion. A second passage was performed when the cells again reached confluence [>70%] and cells were harvested, washed and re-suspended using 0.9% NaCl and human serum albumin. Passage 2 [P2]-MSCs were frozen in 10% DMSO by standard techniques. One week before injection in a patient, P2-frozen cells were thawed and cultured for 1 week before final harvest and injection. For this, freshly P3-harvested cells were washed and resuspended at 3.75×10^6 cells/mL in a 75% NaCl 0.9%/25% HAS 20% solution and conditioned in 5 × 2-mL syringes at room temperature. All reagents were certified and validated. In addition, the batch of fetal bovine serum to be used was selected after extensive testing, and was irradiated to ensure removal of all potential viruses. The whole production process was performed under strict GMP [Good Manufacturing Practice] rules.

2.3. Surgical procedure

Surgery was performed under general anaesthesia by a single surgeon expert in perianal fistulizing CD [CC]. As described in the ADMIRE study,[10] seton[s] were initially removed, and curettage of the fistula tract was performed. Half of the dose of MSCs was equally injected at four quadrants along the tract walls and the other half was injected around the internal opening followed by closure with stiches of the internal opening. In case of two fistulae, the total amount was split and the MSCs were injected in equal volumes in each fistula. A total of 3 × 107 MSCs were injected per patient.[10]

2.4. MRI protocol

MRI examinations were performed according to the protocol of the institution using a 1.5-T [Siemens Aera ou Sola] or 3-T [Siemens Vida] machine. The protocol included T2 turbo-spin-echo [TSE] in the three planes, T2 TSE fat-saturated [FS] in the axial and coronal planes, T1 TSE FS before contrast in the axial plane and T1 TSE FS after gadolinium [5–10 min] in the three planes. Axial and coronal sequences were angled parallel and perpendicular to the anal canal. If performed outside our institution, MRI was included in case of T2 and T1 FS sequence before and after gadolinium in the axial plane.

Table 1. Calculation of the degree of fibrosis

Percentage fibrosis	Score
0	0
1–19	1
20–39	2
40–59	3
60–79	4
80–99	5
100	6

2.5. Clinical assessment

Safety was assessed at each scheduled visit. Any event potentially related to the surgical procedure was recorded including local bleeding, pain, fever, and worsening of perianal disease inclusive as a new abscess.

Fistula closure was defined by the closure of all external openings at clinical examination without discharge at digital pressure and no collection >2 cm at the pelvic MRI. Partial closure was defined by the closure of some, but not all, external openings at clinical examination and no collection >2 cm at the pelvic MRI.[10] 'Super closure' was defined as fistula closure and absence of discharge reported by the patient during the week before the clinical visit.

Table 2. Patients' characteristics

Patients' characteristics at the time of MSC injection, *n* = 16	***N* [%] or median [IQR]**
Female gender	8 [50]
Age [years]	49 [40–58]
Active smoking	5 [33.3]
Previous IBD resection	9 [60]
BMI [kg/m^2]	24.5 [22.5–25.7]
Montreal classification [*n* = 15]	
L1	9 [60]
L2	2 [13]
L3	4 [2]
B1	5 [25]
B2	9 [56.3]
B3	3 [18.8]
Rectal involvement	3 [18.3]
Number of biologics line 0/1/2/3 [*n* = 14]	1 [7.1]/3 [21.4]/9 [64.3]/1 [7.1]
Currents biologics	
No biologic	2 [12.5]
IFX 1×/8 weeks	3 [18.8]
IFX > 1×/8 weeks	1 [6.3]
ADA every other week	5 [31.3]
ADA weekly	2 [12.5]
VDZ	2 [12.5]
UST	0 [0]
Current thiopurine	5 [31.3]
CDAI	97.5 [61.9–126]
PRO stools	2 [0.5–3.0]
PRO pain	0 [0.0–0.8]
SHS	10 [4–14.5]
CRP [mg/I]	1.3 [1.1–6.7]
Faecal calprotectin [µg/g]	111 [42–382]
Perianal CD duration [years]	8 [3–17]
Number of external openings	
1	10 [62.5]
2	6 [37.5]
Surgical closure attempt [*n* = 14]	3 [20]
Time since seton placement [months]	16 [9–22]
Faecal calprotectin [µg/g]	111 [42–382]

Table 3. MRI characteristics at baseline including the detailed Van Assche index and the detailed MAGNIFI-CD index.

MRI characteristics at baseline [*n* = 16]	
Total MAGNIFI-CD index [median, IQR]	
MAGNIFI-CD subitems [number, %]	16.0 [12–22]
Number of external openings	
1	10 [62.5]
2	6 [37.5]
Hypersensitivity primary tract on post-contrast T1-weighted images	
Absent/mild	7 [43.8]
Pronounced	9 [56.2]
Fistula length	
<2.5 cm	1 [6.3]
2.5–5 cm	5 [31.2]
>5 cm	10 [62.5]
Dominant feature	
Fibrous	8 [50]
Granulomatous	4 [25]
Fluid/pus	4 [25]
Extension	
Absent	3 [18.7]
Horseshoe	1 [6.3]
Infralevatoric/ supralevatoric	12 [75]
Inflammatory mass	
Absent	8 [50]
Focal	0 [0]
Diffuse	3 [18.7]
Small collection	2 [12.5]
Medium collection	2 [12.5]
Large collection	1 [6.3]
Total Van Assche index [median, IQR]	11.0 [7–16]
Van Assche index subitems [number, %]	
Number of fistula tracts	
Single unbranched	4 [25]
Single branched	7 [43.8]
Multiples	45 [31.2]
Extension	
Horseshoe	1 [6.3]
Infralevatoric	9 [68.7]
Supralevatoric	4 [25]
Location	
Submucosal	0 [0]
Intersphincteric	2 [12.5]
Transsphincteric	12 [75]
Extrasphincteric	0 [0]
Suprasphincteric	2 [12.5]
Hyperintensity T2-weighted images	
Absent	2 [12.5]

Table 3. Continued

MRI characteristics at baseline [*n* = 16]	
Mild	3 [18.8]
Pronounced	11 [68.7]
Rectal wall involvement	
Absent	6 [37.5]
Thickened	7 [43.5]
Increased signal intensity	8 [50]
Recto-vaginal tract	0 [0]
Inflammatory mass	
Absent	6 [37.5]
Focal	1 [6.3]
Diffuse	3 [18.7]
Small collection	2 [12.5]
Medium collection	2 [12.5]
Large collection	1 [6.3]
Hyperintensity on T1 post-contrast [*n* = 15]	
Absent	5 [33.3]
Mild	6 [40]
Pronounced	4 [26.7]
Dominant feature	
Fibrous	6 [37.6]
Granulomatous	5 [31.2]
Fluid/pus	5 [31.2]
Additional characteristics [median, IQR]	
Fistula+C/Fistula-C	0.5 [0.4–0.5]
Fistula+C/Vessel+C	1.25 [0.9–1.6]
Vessel+C/Vessel-C	1.99 [1.8–2.1]
Int Fistula+C	384.0 [264.0–427.0]
Mid Fistula+C	328.0 [285.0–367.0]
Ext Fistula+C	286.0 [219.0–323.0]
Degree of fibrosis 0 – 1 – 2 – 3 – 4 – 5 – 6	0 [0] – 4 [25] – 2 [12.5] – 5 [31.5] – 4 [25] – 1[6.3] – 0 [0]

2.6. MRI assessment

The radiologists were blinded to the clinical evaluation and biomarkers but not to patient identification nor to the time-point of the MRI scans. The MAGNIFI-CD index[13] and the Van Assche index[12] were evaluated for each patient at baseline, and at weeks 12 and 48. The degree of fibrosis of the fistula tract was evaluated by two radiologists with respectively 5 years [RPG] and 20 years [RMG] of experience. Discrepancies in the degree of fibrosis evaluation were resolved by the intervention of a third abdominal radiologist with 35 years of experience [PM]. Fibrosis has been defined as a hyposignal on T2 TSE without fat suppression [Fat Sat] sequences and a homogenous contrast uptake on T1 Fat Sat late phases. To define the degree of fibrosis of the fistula tract, the entire tract was considered. In case of heterogeneous fibrosis within a single tract, an average value of the fibrosis at different locations was calculated by the radiologists. Six degrees of fibrosis were described [Table 1].[14] The signal intensity of the fistula tract before [Fistula-C] and after [Fistula+C] enhancement was evaluated at three levels (close to external orifice [ext fistula], middle portion [mid fistula] and close to internal orifice [int fistula]). The ratio of signal intensity of the fistula tract before and after contrast was evaluated [Fistula+C/Fistula-C]. Signal intensity of a neighboring vessel [same plane and side] at the same 3 levels of the tract was measured. The signal intensity of the fistula tract before [Vessel-C] and after [Vessel+C] enhancement was evaluated. The ratio of signal intensity of the vessels before and after contrast was calculated [Vessel+C/Vessel-C]. The ratio of fistula signal intensity with contrast and vessel signal intensity with contrast was calculated [Fistula+C/Vessel+C]. The signal intensity of the three parts of the fistula with contrast [ext fistula+C, mid fistula+C, int fistula+C] was separately analysed at each time point. The subgroup of patients with absence of fluid according to the MAGNIFI-CD index and fibrosis of the fistula tract of ≥80% was specifically described.

2.7. Statistical analysis

Results are presented as medians and interquartile range [IQR] for quantitative variables or as numbers [%] for qualitative variables. Some parameters were log-transformed for statistical tests. The evolution of MRI characteristics after bmMSC injection was analysed by a general linear mixed model [GLMM] for global scores and by an ordinal logistic regression model with repeated measures for subscores. The association between clinical outcomes with respect to clinical and radiological factors was tested by the logistic regression model. Odds ratios [ORs] and 95% confidence intervals [CI] were reported. In some cases, the Firth correction was applied. The agreement between radiologists for the interpretation of the degree of fibrosis was measured by Cohen's kappa coefficient and the corresponding 95% CI. Results were considered significant at the 5% level [$p < 0.05$]. Calculations were done with SAS version 9.4 and figures were created in R version 4.1.0.

3. Results

3.1. Baseline characteristics

Eighteen patients were screened for inclusion. One patient was excluded the week before MSC injection because of a new pelvic abscess requiring urgent drainage. The fistula tract could not be catheterized during the surgical procedure in the second excluded patient. Sixteen patients were finally included in the study (eight females, median age 42 years [IQR: 40–58]). Thirteen out of 16 patients were on biologic therapy and 3/16 patients were on monotherapy with immunomodulators at the time of MSC injection. The median CDAI was 97.5 [IQR: 20.0–171.0], median C-reactive protein [CRP] was 1.3 mg/L [IQR: 0.5–42.0] and median faecal calprotectin was 111.0 µg/g [IQR: 26.0–647.0]. The median duration of perianal CD was 8 months [IQR: 8–28]. At baseline, ten [62.5%] and six patients [37.5%] had one and two external openings at clinical examination respectively. The baseline characteristics of the patients are detailed in Table 2 and the Van Assche and MAGNIFI-CD index at baseline in Table 3.

3.2. Safety and efficacy of bmMSC injection

Eleven patients reported no side effects throughout the study. Three patients reported mild pain, one patient reported mild

bleeding and one patient reported increased discharge the week after the MSC injection. The evolution was favourable without intervention except for the patient with discharge requiring transient IFX optimization to every 4 weeks. At 12 weeks, 9/16 patients had fistula[e] closure whereas 6/16 patients had a super closure and 11/16 patients had at least partial closure of their fistulae. Five patients [31.3%] had no closure of the external opening[s]. At week 48, 8/16 patients had a fistula[e] closure. Super closure and at least partial closure were observed in 6/16 and 11/16 patients respectively. Five patients [31.3%] had no closure of the external opening[s] at week 48. These results are illustrated in Figure 1. A decrease of PDAI was observed 48 weeks after bmMSC injection without reaching significance (baseline: 5 [IQR: 2–6], week 48: 3 [IQR: 1–6], $p = 0.06$). No significant modification of the short health scale was observed 48 weeks after bmMSC injection [$p = 0.94$].

3.3. MRI characteristics of the fistula tract post-bmMSC injection

MRI evolution could not be analysed for one patient due to missing injected sequences at weeks 12 and 48 [exams performed in another hospital] and the MRI at week 12 could not be analysed for a second patient for similar reasons. The MAGNIFI-CD index and the Van Assche index did not change significantly after MSC injection with a median MAGNIFIC-CD index at baseline, week 12 and week 48 of 16.0 [IQR: 11.3–19], 15.5 [IQR: 9–24] and 15.0 [IQR: 3.0–19.0] respectively [$p = 0.52$] and a median Van Assche index at baseline, week 12 and week 48 of 16.0 [IQR: 9.0–24.0], 10.0 [IQR: 3.0–17.0] and 9.0 [IQR: 3.0–20.0] respectively [$p = 0.65$]. The individual evolution of the Van Assche and the MAGNIFI-CD index are illustrated in Figure 2. The sub-items of the MAGNIFI-CD index and Van Assche index did not change significantly over time except a trend for a decrease

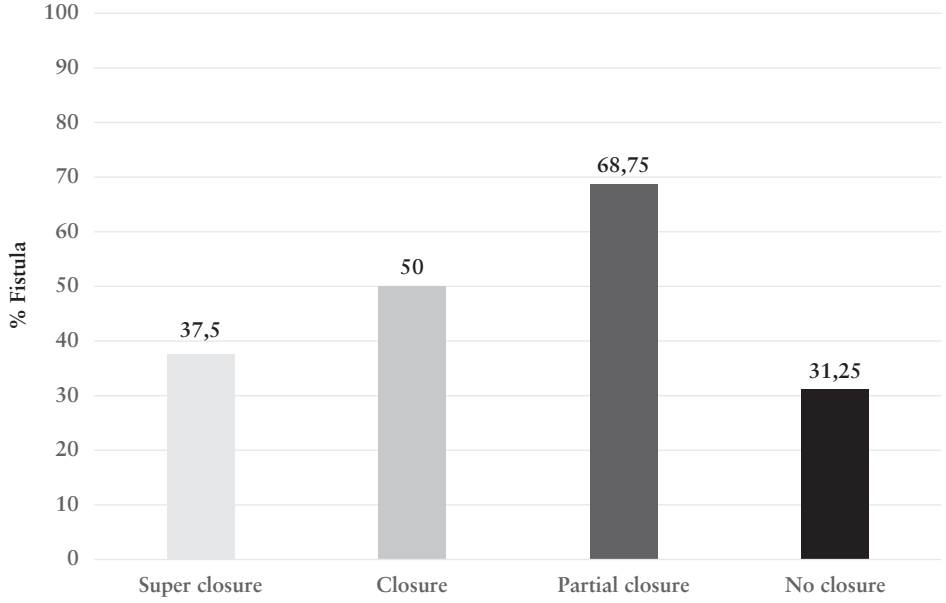

Figure 1. Clinical evolution of perianal fistula including 'super closure', closure, at least partial closure and no closure 48 weeks after bmMSC injection.

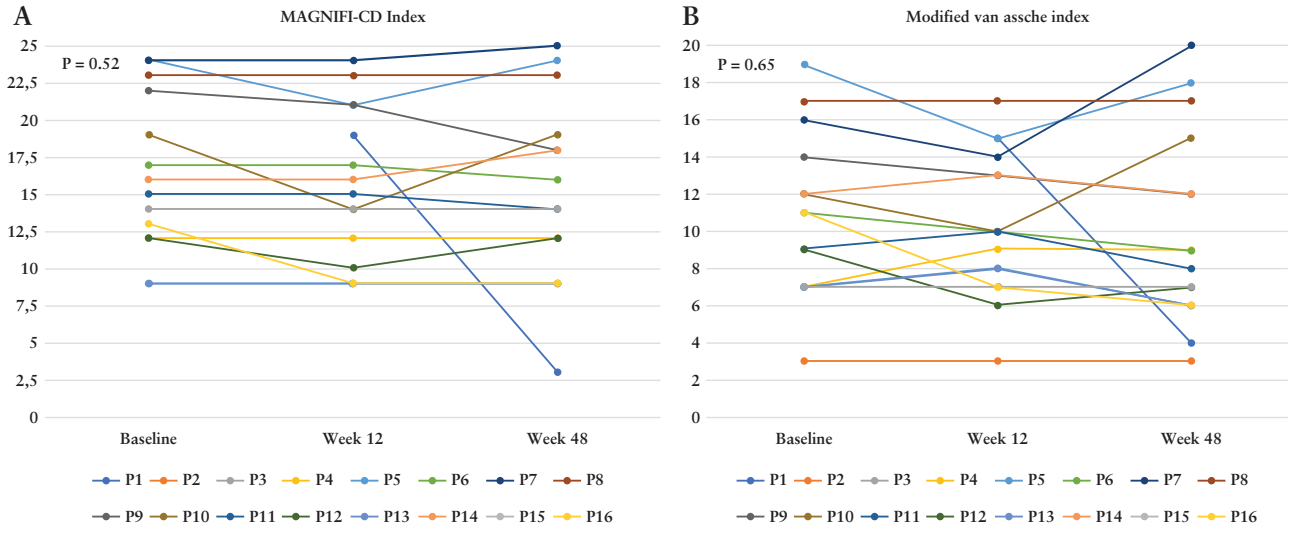

Figure 2. Evolution per patient of the modified Van Assche Index [A] and the MAGNIFI-CD index [B] after bmMSC injection.

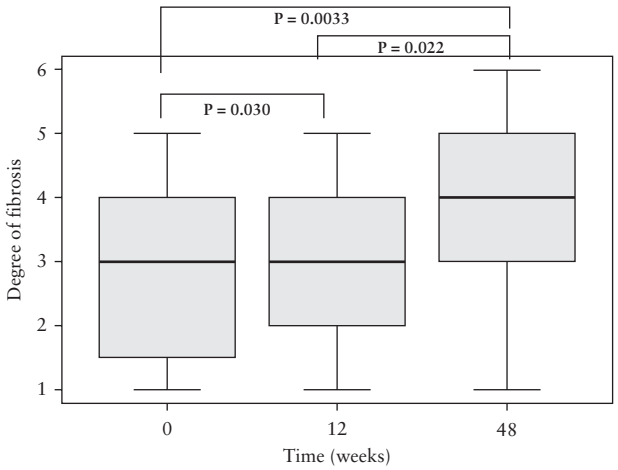

Figure 3. Evolution of the degree of fibrosis of the fistula 12 weeks and 48 weeks after bmMSC injection.

of the MAGNIFI subscore inflammatory mass [$p = 0.056$] and a decrease of the Van Assche subscore hyperintensity of T2-weighted images [$p = 0.094$]. The median degree of fibrosis of the fistula tract at baseline was 3 [IQR: 1–5]; it increased significantly at weeks 12 and 48 after MSC injection [Figure 3]. The ratio of contrast enhancement fistula/vessel [fistula+C/vessel+C] decreased significantly from baseline to week 48 after MSC injection [$p = 0.008$]. When specifically analysing the enhancement of the different portions of the fistula tract, the enhancement of the ext fistula [$p = 0.001$], mid fistula [$p = 0.001$] and int fistula [$p < 0.001$] decreased significantly at week 48 compared to baseline. In the subgroup of patients with absence of fluid according to the MAGNIFI-CD index and a fibrosis of the fistula tract of ≥80%, 1/16 patients met these criteria at the baseline compared to three and five patients at weeks 12 and 48 respectively. Statistical analysis demonstrated a significant association between favourable RMN outcome [absence of fluid and fibrosis of the fistula tract of ≥80%] and super closure [$p = 0.036$, OR = 18, 95% CI 1.2–260.9]. The evolution of the different parameters of

Table 4. Evolution of MRI characteristics after bmMSC injection.

MRI characteristics [median, IQR]	Baseline	W12	W48	*p*
Total MAGNIFI-CD Index	16.0 [12.0–22.0]	15.5 [11.0–20.5]	15.0 [10.5–19.5]	0.52§
MAGNIFI-CD Index sub-items				
Number of fistula tracts	2 [1.5–2.0]	2 [1.5–2.0]	2 [1.0–2.0]	1.00#/0.14*
Hyperintensity primary tract on post-contrast T1-weighted images	1.0 [0.0–1.0]	0.0 [0.0–1.0]	1.0 [0.0–1.0]	0.14#/0.70*
Dominant feature	1.0 [0.0–2.0]	1.0 [0.0–1.5]	0.5 [0.0–1.5]	0.14#/0.21*
Fistula length	2.0 [1.5–2.0]	2.0 [1.0–2.0]	2.0 [1.0–2.0]	0.31#/0.13*
Extension	2.0 [2.0–2.0]	2.0 [2.0–2.0]	2.0 [1.5–2.0]	1.00#/0.30*
Inflammatory mass	1.5 [0.0–3.5]	1.0 [0.0–3.0]	1.0 [0.0–3.0]	0.056#/0.32*
Total modified Van Assche Index	11.0 [7.0–16.0]	10.0 [7.5–14.5]	9.0 [6.5–16.0]	0.65§
Modified Van Assche Index sub-items				
Number of fistula tracts	2.0 [1.5–3.0]	2.0 [1.5–3.0]	2.0 [1.5–2.5]	1.00#/0.56*
Location	2.0 [2.0–2.0]	2.0 [2.0–2.0]	2.0 [2.0–2.0]	1.00#/1.00*
Extension	1.0 [1.0–2.5]	1.0 [1.0–2.5]	1.0 [1.0–2.5]	0.30#/0.98*
Hyperintensity T2-weighted images	2.0 [1.0–2.0]	2.0 [0.5–2.0]	1.5 [0.0–2.0]	0.54#/0.094*
Rectal wall involvement	1.0 [0.0–2.0]	1.0 [0.0–2.0]	0.5 [0.0–2.0]	0.15#/0.067*
Recto-vaginal tract	0.0 [0.0–0.0]	0.0 [0.0–0.0]	0.0 [0.0–0.0]	1.00#/1.00*
Inflammatory mass	1.0 [0.0–3.5]	1.0 [0.0–3.0]	0.5 [0.0–3.5]	0.25#/0.36*
Hyperintensity on T1 post-contrast	1.0 [0.0–2.0]	1.0 [0.0–1.0]	1.0 [0.0–1.0]	0.73#/0.50*
Dominant feature	1.0 [0.0–2.0]	1.0 [0.0–1.0]	0.5 [0.0–1.5]	0.27#/0.20*
Additional characteristics				
Fistula+C/Fistula-C	0.5 [0.44–0.53]	0.56 [0.46–0.6]	0.55 [0.50–0.61]	0.27§
Fistula+C/Vessel+C	1.25 [0.92–1.55]	1.17 [0.90–1.3]	0.96 [0.79–0.38]	0.0084§
Vessel+C/Vessel-C	1.99 [1.82–2.08]	1.98 [1.61–2.54]	1.94 [1.74–2.13]	0.66§
Ext Fistula+C	286.0 [219.0 –323.0]	300.0 [217.0–345.0]	188.5 [144.0–260.0]	0.0020§
Mid Fistula+C	328.0 [285.0–367.0]	434.0 [244.0 –434.0]	221.0 [141.0–295.5]	0.0013§
Int Fistula+C	384.0 [264.0–427.0]	437.0 [312.0 –637.0]	198.5 [138.5 –246.0]	<0.0001§
Degree of fibrosis	3.00 [1.50 –4.0]	3.00 [2.0–4.0]	4.00 [3.0–5.0]	0.030#/0.0033*

The *p* values [§] represent the global *p*-value of the GLMM test.
The other *p*-values [# and *] are given by the ordinal logistic regression model with repeated measures, and the *p*-values are respectively the comparisons between week 12 and baseline [#] and between week 48 and baseline [*].

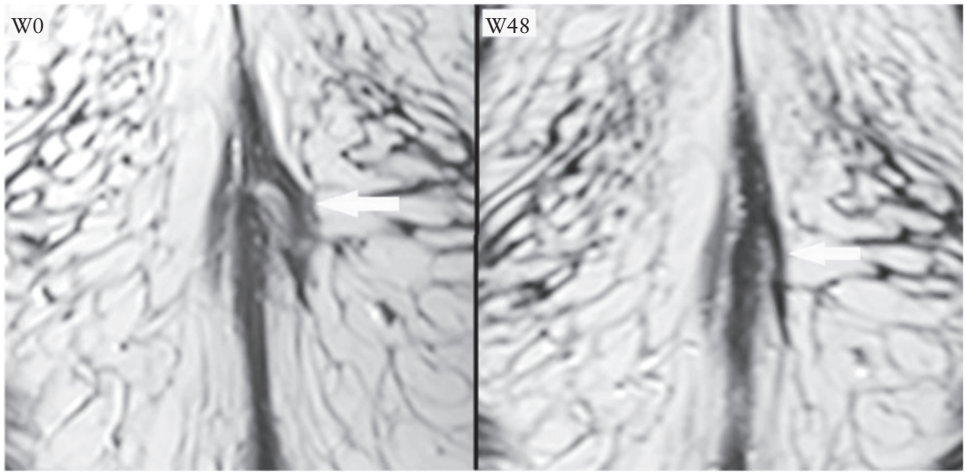

Figure 4. Illustration of a favourable evolution of the perianal fistula tract 48 weeks after bmMSC injection on T2 Turbo Spin Echo without fat suppression sequence in axial plan. At baseline the degree of fibrosis was between 40 and 59%. At week 48 the tract was completely fibrotic.

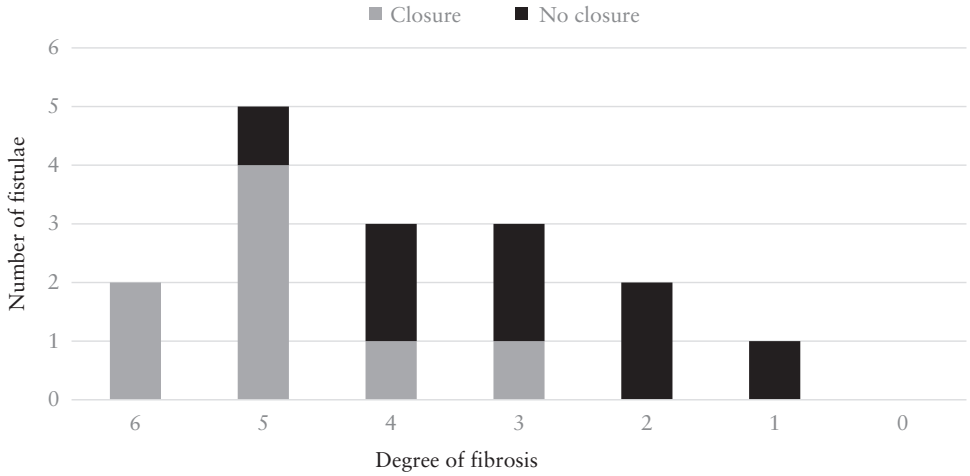

Figure 5. Degree of fibrosis correlated with fistula closure at week 48.

the pelvic MRI are summarized in Table 4. Figure 4 illustrates a favourable evolution of the perianal fistula after bmMSC injection.

3.4. MRI fistula tract characteristics in relation to fistula closure after MSC injection

The MAGNIFI-CD index and Van Assche index at weeks 12 and 48 did not differ between patients with fistula closure or not. The relationship between fistula closure and fistula tract fibrosis is illustrated in Figure 5. A lower MAGNIFI-CD index was observed in patients with fistula 'super' closure at week 12 compared to patients without 'super' closure [$p = 0.044$, OR = 1.4, 95% CI = 1.01–1.96]. The degree of fibrosis increased significantly after MSC injection in patients with fistula closure at week 48 compared to patients with no fistula closure [$p = 0.036$, OR = 4.2, 95% CI = 1.1–16.5]. All seven patients except one showing a percentage of fibrosis >80% [degree 5 or 6] at week 48 had fistula closure whereas all patients with a percentage of fibrosis <40% [degree < 3] at week 48 had no fistula[e] closure [Figure 5]. In the subgroup of patients with absence of fluid at MRI and a fibrotic tract of ≥80% [$n = 5$], 80% of the patients had also a super closure.

Several other parameters of pelvic MRI at week 48 were associated with a favourable clinical outcome including fistula closure or 'super closure' at week 48. MAGNIFI-CD subitems associated with 'super' were low number of fistula tracts [one vs two or three, $p = 0.02$, OR = 45.5, 95% CI = 2.27–100], hyperintensity of primary tract on post-contrast T1-weighted images [$p = 0.027$, OR = 0.05, 95% CI = 0.04–0.71], and short fistula length [$p = 0.017$, OR = 37.0, 95% CI = 1.89–100]. Van Assche index factors associated with super closure at week 48 were hyperintensity T2-weighted images [$p = 0.019$, OR = 0.085, 95% CI = 0.01–0.67] and hyperintensity on T1 post-contrast [$p = 0.032$, OR = 0.03, 95% CI = 0.01–0.59]. The factors associated with fistula [super] closure are illustrated in Table 5.

3.5. Predictive factors of fistula closure after bmMSC injection

No demographic factors, CD characteristics, including perianal CD duration and time since seton placement, nor current CD medications were predictive of fistula closure or 'super' closure at weeks 12 or 48. Fistula closure at week 12 was predictive of fistula closure at week 48 [$p = 0.024$, OR = 21,

Table 5. Clinical and radiological factors predictive/associated with clinical outcome. Only significant values are reported

	p	OR [95% CI]
Predictive factors of super closure at week 12		
MAGNIFI-CD fistula length baseline	0.046	0.05 [<0.01–0.91]
Associated factors of super closure week 12		
MAGNIFIC-CD index week 12	0.044	0.71 [0.51–0.99]
Predictive factors of closure week 48		
Closure week 12	0.024	21.0 [1.5–293]
Associated factors with closure week 48		
RMN ratio Vessel+C/Vessel-C week 48	0.048	0.008 [<0.01–0.95]
Degree of fibrosis week48	0.036	4.20 [1.1–16.5]
Associated factors with super closure week 48		
MAGNIFI-CD number of fistula tracts week 48	0.012	0.022 [0.01–0.44]
MAGNIFI-CD hyperintensity primary tract on post-contrast T1-weighted images week 48	0.027	0.05 [0.04–0.71]
MAGNIFI-CD fistula length week 48	0.017	0.027 [0.01–0.53]
Modified Van Assche hyperintensity T2-weighted images week 48	0.019	0.085 [0.011–0.67]
Modified Van Assche hyperintensity on T1 post-contrast week 48	0.032	0.03 [0.002–0.59]

95% CI = 1.5–293]. Fistula length <5 cm according to the MAGNIFI-CD index before MSC injection was predictive of fistula 'super' closure at week 12 [$p = 0.045$, OR = 20, 95% CI = 1.1–100]. No other MRI parameters were predictive of fistula closure or 'super' closure at weeks 12 and 48. The predictive factors of fistula [super] closure are detailed in Table 5.

3.6. Clinical evolution of luminal CD post-bmMSC injection

The median CDAI did not change significantly 12 and 48 weeks after bmMSC injections (baseline: 97.5 [IQR: 61.9–125.5], week 12: 73.8 [IQR: 34.8–145.7], week 48: 76.2 [IQR: 49.6–171.6], $p = 0.79$). PROs for stool frequency remained stable [$p = 0.79$] as well as biomarkers including CRP [$p = 0.71$] and faecal calprotectin [$p = 0.13$]. PROs for abdominal pain decreased significantly between baseline and week 48 [$p = 0.011$]. The SHS did not change significantly between 12 and 48 weeks after bmMSC injection [$p = 0.98$]. No correlation was found between the degree of fibrosis and SHS [$p = 0.14$].

4. Discussion

This study shows that the injection of allogenic bmMSCs had a good safety profile for the treatment of perianal fistulae in CD and achieved 50% closure after 48 weeks according to a validated definition including clinal assessment and absence of abscess >2 cm at MRI.[6] The MRI assessment demonstrated that the degree of fibrosis of the fistula tract achieved at week 48 was significantly associated with fistula closure. Other radiological factors including hyperintensity on T2-weighted images and hyperintensity on post-contrast T1-weighted images were associated with a more stringent definition of fistula closure [also including absence of fistula discharge reported by the patient in the week preceding the assessment], suggesting that both the attenuation of the inflammatory component and the increase in fibrosis could be considered as MRI targets in the management of perianal CD.

Our results are comparable to the results of the ADMIRE study regarding safety and efficacy.[10] We did not observe any severe complication of the MSC injection procedure over 1 year of follow-up. As reported in other series or early controlled trials,[11,17–21] this contributes to strengthen the safety profile of MSCs for the treatment of perianal CD fistulae. The efficacy of adipose-derived MSCs was confirmed in several small series[17–19,21] but few studies used allogenic bmMSCs.[11,17,20] Molendijck described for the first time the efficacy of allogenic bmMSCs for the treatment of fistulizing perianal CD in a placebo-controlled pilot study performed over 12 weeks with a small number of patients.[11] The long-term retrospective follow-up of this cohort showed that the beneficial effect of bmMSC therapy on the number of draining fistulas previously reported at week 24 was maintained after 4 years.[22] In our cohort, albeit with a shorter follow-up, the clinical benefit was also maintained over 1 year. Additionally, fistula closure at week 12 was predictive of closure at week 48 [$p = 0.024$, OR = 21] which confirms the long-term maintained benefit. All these studies contribute to suggest an efficacy not only for adipose-derived MSCs but also bmMSCs. However, more in-depth studies to assess the respective properties of these cells for the treatment of perianal fistulizing CD are still needed.

The validated definition of clinical fistula closure is defined as 'closure of all external openings at clinical examination without discharge at digital pressure'.[10] However, patients can report discharge despite closure of the external opening at clinical examination, suggesting episodes of transient opening with secondary re-epithelialization. 'Super closure', a more stringent but not validated definition of clinical closure, was thus explored in this study. In total, 35% of patients reached this definition after both 12 and 48 weeks. More MRI factors were associated with fistula 'super closure' compared to fistula closure, including features associated with inflammation [hyperintensity on T2-weighted images, hyperintensity on post-contrast T1-weighted images] and morphological characteristics [fistula length, number of tracts]. The definition of 'super closure' seems to better reflect the deep healing process in perianal fistulizing CD. Further studies are needed to validate this definition and to confirm its association with a more favourable long-term outcome.

In our study, despite an association between the MAGNIFIC CD index and fistula super closure at week 12, the total

MAGNIFI CD index did not decrease significantly after bmMSC injection. This can be explained by the nature of the subitems that precisely describe the lesions but only slowly evolve over time such as the number of tracts, and the extension and the length of the tract. Of the subitems directly related to inflammation such as T2 hyperintensity, the presence of liquid seems more relevant to evaluate healing of the fistula tract after treatment, and they should be validated specifically in larger cohorts as surrogate markers of fistula healing. Using these subitems in a larger cohort, Van Assche *et al*.[23] reported that active signs of inflammation including T2 hyperintensity decreased in some patients after IFX treatment. These results are comparable to our findings after bmMSC injection. While mucosal and transmural healing are well defined in luminal CD,[4,23] no validated definition of perianal fistula healing is available. Characteristic features associated with long-term clinical fistula closure are still being debated.

Our study assessed for the first time the degree of fibrosis after bmMSC injection and its correlation with clinical outcome. The degree of fibrosis was associated with fistula closure in our study with a 4-fold increased probability of fistula closure at week 48. Van Rijn *et al*.[14] also demonstrated a higher degree of fibrosis after surgical treatment compared to medical treatment, suggesting that curettage and closure of the internal opening play a crucial role in the fistula healing process. This could explain the higher rates of fistula closure after surgical vs medical treatment.[14]

Regarding predictive factors, only a short fistula length at baseline according to the MAGNIFI-CD index was predictive of super closure at week 12. This factor has already been validated in a paediatric retrospective Korean cohort.[24] In our study, MRI features highlighting a low level of inflammation and high degree of fibrosis were associated with fistula super closure after 48 weeks, suggesting that these factors could be part of a potential definition of fistula healing and might represent a target in the management of fistulizing perianal CD. The factors should be validated in larger prospective cohorts.

The main strength of our study is its prospective design with standardized clinical and MRI evaluation allowing precise evaluation of clinical and MRI evolution after bmMSC injection. Another strength of this work is the study of original MRI features that might be more appropriate to precisely assess the evolution of perianal fistulizing CD after treatment. Our study has some limitations, including the small number of patients, that do not allow definitive conclusions on clinical and MRI predictive factors. Our findings should be confirmed in larger cohorts. The absence of a control group in which only curettage of the fistula tract followed by closure of the internal opening would have been performed does not allow any comparison as was performed in the ADMIRE trial in which high response rates were observed in the control group. The majority of studies used adipose-derived MSCs since they are easier to harvest, replicate faster and proliferate longer in culture.[10,18,19,21] There is currently no evidence to promote a specific source of MSCs to treat perianal fistulizing CD. Although no trial comparing the efficacy of the different kinds of MSCs to treat perianal fistulizing CD are available, several studies have demonstrated the efficacy of bmMSCs in this indication.[11,17,20,22] Our choice was supported by a closed collaboration with the Laboratory of Cell and Gene Therapy LTCG of the University of Liège, allowing easy access to the cells and many projects in development.

During this study, samples of biological material from the fistula tract before the injection of bmMSCs were collected during the surgical procedure for further analysis, including to understand of fistula pathogenesis and to identify predictive factors.

In conclusion, our study suggests safety and favourable clinical outcome after the injection of bmMSCs for the treatment of perianal fistulizing CD. The prospective standardized MRI follow-up identified for the first time specific radiological changes after bmMSC injection, including an increased degree of fibrosis. This study also suggests some MRI features that could represent relevant targets in perianal CD to implement a treat-to target strategy.

Funding

No funding was received for this study.

Conflict of Interest

The authors have no disclosures relating to the present study.

Acknowledgments

None.

Author Contributions

CR was involved in the selection of patients, clinical follow-up, acquisition and the analysis of the data, and conception of the manuscript. EL was involved in the design and the conception of the study, selection of patients, clinical follow-up of the patients and reviewing the manuscript. LB was involved in the clinical follow-up and acquisition of the data. CC performed the surgical injection of the bmMST. CL, EB and YB produced the bmMSCs. PM, RMG and RPG analysed the pelvic MRI. All authors critically reviewed and validated the final manuscript.

Data Availability Statement

All the original data generated in the course of the study are incorporated into the article and its online figures.

References

1. Schwartz DA, Loftus EV Jr, Tremaine WJ, Panaccione R, Harmsen WS, Zinsmeister AR, Sandborn WJ. The natural history of fistulizing Crohn's disease in Olmsted County, Minnesota. *Gastroenterology* 2002;**122**:875–80.
2. Hellers G, Bergstrand O, Ewerth S, Holmström B. Occurrence and outcome after primary treatment of anal fistulae in Crohn's disease. *Gut* 1980;**21**:525–7.
3. Kamm MA, Ng SC. Perianal fistulizing Crohn's disease: a call to action. *Clin Gastroenterol Hepatol* 2008;**6**:7–10.
4. Turner D, Ricciuto A, Lewis A, *et al*.; International Organization for the Study of IBD. STRIDE-II: An Update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) Initiative of the International Organization for the Study of IBD (IOIBD): determining therapeutic the goals for treat-to-target strategy in IBD. *Gastroenterology* 2021;**160**:1570–83.
5. Adamina M, Bonovas S, Raine T, *et al*. ECCO guidelines on therapeutics in Crohn's disease: surgical treatment. *J Crohns Colitis* 2020;**14**:155–68.

6. Soltani A, Kaiser AM. Endorectal advancement flap for cryptoglandular or Crohn's fistula-in-ano. *Dis Colon Rectum* 2010 Apr;**53**:486–95.
7. Vidon M, Munoz-Bongrand N, Lambert J, *et al*. Long-term efficacy of fibrin glue injection for perianal fistulas in patients with Crohn's disease. *Colorectal Dis* 2021 Apr;**23**:894–900.
8. van Praag EM, Stellingwerf ME, van der Bilt JDW, Bemelman WA, Gecse KB, Buskens CJ. Ligation of the intersphincteric fistula tract and endorectal advancement flap for high perianal fistulas in Crohn's disease: a retrospective cohort study. *J Crohns Colitis* 2020;**14**:757–63.
9. Sands BE, Blank MA, Patel K, van Deventer SJ; ACCENT II Study. Long-term treatment of rectovaginal fistulas in Crohn's disease: response to infliximab in the ACCENT II Study. *Clin Gastroenterol Hepatol* 2004;**2**:912–20.
10. Panés J, García-Olmo D, Van Assche G, *et al*; ADMIRE CD Study Group Collaborators. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial. *Lancet* 2016;**388**:1281–90.
11. Molendijk I, Bonsing BA, Roelofs H, *et al*. Allogeneic bone marrow derived mesenchymal stromal cells promote healing of refractory perianal fistulas in patients with Crohn's disease. *Gastroenterology* 2015;**149**:918–27.e6.
12. Van Assche G, Vanbeckevoort D, Bielen D, *et al*. Magnetic resonance imaging of the effects of infliximab on perianal fistulizing Crohn's disease. *Am J Gastroenterol* 2003;**98**:332–9.
13. Hindryckx P, Jairath V, Zou G, *et al*. Development and validation of a magnetic resonance index for assessing fistulas in patients with Crohn's disease. *Gastroenterology* 2019;**157**:1233–1244.
14. van Rijn KL, Meima-van Praag EM, Bossuyt PM, *et al*. Fibrosis and MAGNIFI-CD activity index at MRI to predict treatment outcome in perianal fistulising Crohn's disease patients. *J Crohns Colitis*. 2022;**16**:708–16. doi:10.1093/ecco-jcc/jjab168.
15. Lechanteur C, Briquet A, Giet O, Delloye O, Baudoux E, Beguin Y. Clinical-scale expansion of mesenchymal stromal cells: a large banking experience. *J Transl Med* 2016;**14**:145.
16. Gregoire C, Briquet A, Pirenne C, Lechanteur C, Louis E, Beguin Y. Allogeneic mesenchymal stromal cells for refractory luminal Crohn's disease: a phase I-II study. *Dig Liver Dis* 2018;**50**:1251–5.
17. López-García A, Rovira M, Jauregui-Amezaga A, *et al*. Autologous haematopoietic stem cell transplantation for refractory Crohn's disease: efficacy in a single-centre cohort. *J Crohns Colitis* 2017;**11**:1161–8.
18. Dige A, Hougaard HT, Agnholt J, *et al*. Efficacy of injection of freshly collected autologous adipose tissue into perianal fistulas in patients with Crohn's disease. *Gastroenterology* 2019;**156**:2208–2216.e1.
19. Cho YB, Lee WY, Park KJ, Kim M, Yoo H-W, Yu CS. Autologous adipose tissue-derived stem cells for the treatment of Crohn's fistula: a phase I clinical study. *Cell Transplant* 2013;**22**:279–85.
20. 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**:788–98.
21. 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.
22. Barnhoorn MC, Wasser M, Roelofs H, *et al*. Long-term evaluation of allogeneic bone marrow-derived mesenchymal stromal cell therapy for Crohn's disease perianal fistulas. *J Crohns Colitis* 2020;**14**:64–70.
23. Van Assche G, Vanbeckevoort D, Bielen D, *et al*. Magnetic resonance imaging of the effects of infliximab on perianal fistulizing Crohn's disease. *Am J Gastroenterol* 2003;**98**:332–9.
24. Kim PH, Kim SH, Cho YA, *et al*. Ability of pelvic magnetic resonance imaging to predict clinical course of perianal fistula in paediatric Crohn's disease patients. *J Crohns Colitis* 2021;**15**:1152–60.