Stromal cells in inflammatory bowel disease : perspectives of local mesenchymal stromal cell therapy

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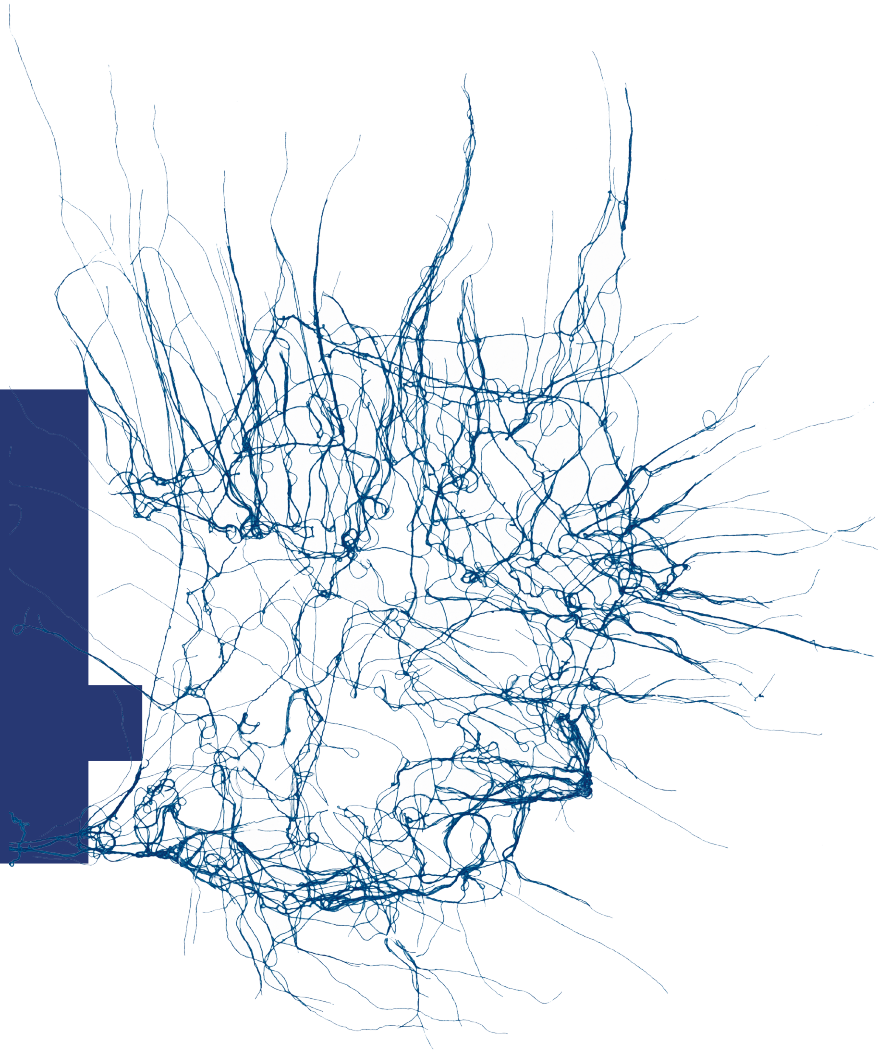

LONG-TERM EVALUATION OF ALLOGENEIC BONE MARROW-DERIVED MESENCHYMAL STROMAL CELL THERAPY FOR CROHN'S DISEASE PERIANAL FISTULAS

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ABSTRACT

Background & Aims

The long-term safety and efficacy of allogeneic bone marrow-derived mesenchymal stromal cell (bmMSC)-therapy in perianal Crohn's disease (CD) fistulas is unknown. We aimed to provide a 4-year clinical evaluation of allogeneic bmMSC treatment of perianal CD fistulas.

Methods

A double-blind dose-finding study for local bmMSC-therapy in 21 patients with refractory perianal fistulising Crohn's disease was performed at the Leiden University Medical Center in 2012-2014. All patients treated with bmMSCs ($1x10^7$ bmMSCs cohort 1, n=5; $3x10^7$ bmMSCs cohort 2, n=5; $9x10^7$ bmMSCs cohort 3, n=5) were invited for a 4-year evaluation. Clinical events were registered, fistula closure was evaluated and anti-human leukocyte antigen (HLA) antibodies were assessed. Patients were also asked to undergo a pelvic MRI scan and rectoscopy.

Results

Thirteen out of 15 patients (87%) treated with bmMSCs were available for long-term follow-up. Two non-MSC related malignancies were observed. No serious adverse events thought to be related to bmMSC-therapy were found. In cohort 2 (n=4) all fistulas were closed 4 years after bmMSC-therapy. In cohort 1 (n=4) 63% and cohort 3 (n=5) 43% of the fistulas were closed. In none of the patients anti-HLA antibodies could be detected 24 weeks and 4 years after therapy. Pelvic MRI scans showed significantly smaller fistula tracts after 4 years.

Conclusions

Allogeneic bmMSC-therapy for CD associated perianal fistulas is also on the long-term a safe therapy. In bmMSC treated patients, fistulas with closure at week 24 were still closed after 4 years. (ClinicalTrials.gov ID NCT01144962)

INTRODUCTION

A serious, often persistent, complication of Crohn's disease (CD), occurring in 25% of the patients, is the development of perianal fistulas[1, 2]. Perianal fistulas are ulcer tracts that connect the intestinal lumen, usually from the anal canal or rectum, with the perianal skin and are associated with a strongly impaired quality of life. Although healing of luminal ulcers can be achieved, complete fistula healing in CD is difficult and is accompanied by multiple relapses. The combination of both biological therapies and fistula drainage with a non-cutting seton is still the cornerstone of treatment. After adequate drainage, closure of the tract can be performed using the advancement flap or the ligation procedure of the inter sphincteric tract. Fecal diversion is considered one of the last treatment options with a first response rate of 64%[3]. Of the biological therapies, only infliximab and adalimumab have been found effective in randomized controlled trials for the closure of perianal fistulas in CD till now[4-6]. In the end, only 37% of the patients with complex perianal fistulas showed fistula closure after a median follow-up of 10 years using combined medico-surgical therapies[7]. These disappointing healing rates show the need for new therapies of perianal fistulizing CD.

A promising therapy for perianal CD fistulas is the local injection of bone marrow-derived mesenchymal stromal cells (bmMSCs)[8]. In 2012-2014 we conducted a randomized placebo-controlled dose-finding study for the treatment of perianal CD fistulas with allogeneic bmMSCs[9]. That study, with a follow-up of 24 weeks, showed that locally administrated bmMSC-therapy for perianal CD fistulas was safe and feasible. Furthermore, the study showed a significant improvement of fistula closure in patients treated with $3x10^7$ bmMSCs (cohort 2) compared with placebo treated patients, with a reduction in the number of draining fistulas of 86%. These promising results have been confirmed in a large multicenter trial from Panes et al[10]. In that study, fistula closure was reached in 50% (n=53) of the 107 patients receiving local adipose-tissue derived MSCs (Cx601; $12x10^7$ MSCs) versus 34% (n=36) in the 105 placebo treated patients at week 24. In 2017, Cx601 was approved by the European Medicines Agency for the treatment of complex perianal CD. Here we report on long-term safety and efficacy of local bmMSC-therapy in CD perianal fistulas.

MATERIALS AND METHODS

Study design

We asked all patients enrolled in the randomized placebo-controlled dose-finding trial 'Allogeneic Bone Marrow Derived Mesenchymal Stem Cells for the Treatment of Fistulas in Patients with Refractory Perianal Crohn's Disease' [NCT01144962; clinicaltrials.gov]

for a 4-years follow-up evaluation. Full details of the original study design, the patient eligibility criteria, and the primary outcome of the study after 24 weeks of follow-up have been published previously[9] (Supplementary Figure 1). In short, 21 patients with refractory perianal fistulizing CD were enrolled. Patients were double-blind randomized in a 5:2 fashion to receive locally either $1x10^7$ (cohort 1, n=5), $3x10^7$ (cohort 2, n=5) or $9x10^7$ (cohort 3, n=5) bmMSCs or 0.9% NaCl/5% human albumin solution with no cells (placebo group, n=6). Before local bmMSC or placebo injection the surgeon performed curettage of the fistula tract(s), trimming of the mucosa and skin of respectively the internal and external opening and closure of the internal opening with an absorbable polydioxanone II 4/0 suture. Subsequently, half of the bmMSCs or placebo suspension was injected via the anus in the fistula wall around the closed internal opening. The second half was injected in the wall as close as possible to the internal opening by introducing the syringe into the fistula tract via the external opening.

Four years after treatment in the clinical trial, patients who received bmMSC-therapy were asked to visit the outpatient clinic and placebo treated patients were consulted by phone. Patients treated with bmMSCs were asked for clinical events and the clinical fistula closure was evaluated (e.g. no fistula discharge). Furthermore, patients were asked to fill out questionnaires, concerning current medication use, operation history, the Perianal Disease Activity Index (PDAI), adapted Vaizey fecal incontinence score, Crohn's Disease Activity Index (CDAI), Short Form (SF)-36 score and Short Inflammatory Bowel Disease Questionnaire (sIBD-Q). The CDAI and Vaizey score were not calculated in two patients with a stoma. All bmMSC treated patients were also asked to undergo a rectoscopy and pelvic MRI 4 years after MSC-therapy. Pelvic MRI scans before bmMSC-therapy and 4-years after therapy were evaluated by an experienced radiologist (M.N.J.M.W.). The diameter of the fistula tract(s) and the presence of collections were reported. Improvement on MRI was defined by fistulas containing less fluid compared with the MRI scan made before bmMSC-therapy. Furthermore, if possible, blood was drawn for standard measurements and serum collection.

The original study and the amendment for this follow-up study were approved by the Central Committee on Research involving Human Subjects and the local Medical Ethical Committee of the Leiden University Medical Center. All patients gave a renewed written informed consent for the follow-up study. Data were collected between May 2016 and May 2018. All authors had access to the study data and reviewed and approved the final manuscript.

HLA-antibody measurements

In the serum of patients treated with bmMSCs, donor-specific antibodies against HLA class I and II were measured using the Luminex Screening Assay: Lifecodes Lifescreen Deluxe (LMX) kit according to the manufacturer's manual (Immucor Transplant Diagnostics Inc., Stamford, CT, USA); the modified protocol as described by Kamburova et al[11] was used. Provider suggested definitions of the negative and positive discriminations were used. When positive, donor specificity was determined by single antigen bead assay (Luminex single-antigen (LSA), Lifecodes) according to the manufacturers protocol. Sera were pre-treated with EDTA before testing in LSA. All Luminex testing were analyzed with a Luminex 100 reader. First the serum collected 24 weeks (from two patients only week 12 serum was available) after bmMSC-therapy was measured. When no antibodies were found after 24 weeks, serum samples 4 years after bmMSC-therapy were tested when available.

Statistical analysis

Paired data (before and 4 years after bmMSC-therapy) were compared using the paired sample t-test. Data were analysed using SPSS statistical software package (version 23, IBM SPSS Statistics for Windows, IBM Corp, Armonk, NY) or GraphPad Prism software (version 7, San Diego, CA) and expressed as means ± SEM. $P \leq .05$ was considered statically significant.

RESULTS

Study population

Two of the 15 bmMSCs treated patients were not available for long-term follow-up (Table 1). One patient in cohort 1 died due to an adenocarcinoma in the cecum, which was already described in the original paper of the study[9] and in cohort 2 one patient was lost to follow-up. Six patients received placebo, of whom 2 patients received open-label bmMSC-therapy in our center 2 years after the initial study and 1 patient was treated with Cx601[10] 2 years later. These 3 patients had draining fistula(s) at the time of these treatments. The other 3 placebo treated patients were consulted by phone for evaluation of fistula drainage. Medication use at the time of the follow-up visit and surgery in the past 4 years are described in Table 2.

Safety

Clinical events over 4 years after bmMSC-therapy were assessed. Four patients had developed perianal abscesses, 3 patients had CD activity in the last 4 years and 5 patients were treated for infections (Table 3). During the 4-year follow-up visit we detected a B-cell lymphoproliferative disease (LPD) in the rectum of a patient treated with $3x10^7$ bmMSCs

(cohort 2) as reported previously[12]. To investigate whether bmMSC-therapy contributed to this LPD, we analyzed the possibility of EBV transfer via the MSC product and the persistence of bmMSCs in the LPD tissue using short tandem repeat analysis. Since no EBV-DNA was detected in the bmMSC-product and no cells containing the DNA of the MSC donor were detected in the lymphoproliferative lesion, it was concluded that that a relation between this EBV-associated LPD and the bmMSC-therapy was unlikely, but rather the result of prolonged immunosuppressive therapy.

TABLE 1. Study population. †1 patient was consulted by phone due to emigration. ‡ 1 patient was consulted by phone due to long travel time. § all with placebo treated patients were consulted by phone but were not included in follow-up analysis. ¶ in patients included in the follow-up.

Cohort	bmMSCs	Treated patients (n)	Patients in follow-up (n)	Original treated fistulas (n)¶	MRI (n)	Rectoscopy (n)
1	$1x10^7$	5	4	8	3	3
2	$3x10^7$	5	4†	6	3	2
3	$9x10^7$	5	5‡	7	3	1
Placebo	-	6	3§	3	-	-

Efficacy

The beneficial effect of bmMSC-therapy on the number of draining fistulas previously reported at week 24 was maintained after 4 years (Figure 1 and Supplementary Table 1). In cohort 1, 75% [3/4] of the patients had complete clinical fistula closure after 4 years, as determined by the absence of discharge. In cohort 2, all patients (100% [4/4]) had complete clinical fistula closure after 4 years. In contrast, in cohort 3, only 1 (20% [1/5]) patient had clinical fistula closure, however 2 patients showed partial fistula closure, with closure of either 1 of their 2 fistulas (40% [2/5]). In 2 patients (cohort 2 and 3) perianal fistulas closed between week 24 and the 4-year follow-up without surgical interventions in the perianal region. In the 3 patients in the placebo group, none of the patients experienced partial or complete fistula closure at 4 years (0% [0/3]). The bmMSCs from one donor (bmMSC-B) showed lower fistula healing rates compared with the bmMSCs of the other 4 MSC donors (Supplementary Figure 2).

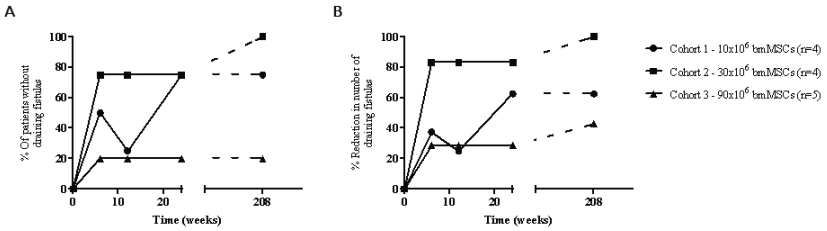

FIGURE 1. Fistula closure after 4 years of follow-up. A. Percentage of patients per group without draining fistulas at week 6, 12, 24 and 208 weeks after therapy. B. Percentage of reduction in the number of draining fistulas per group at week 6, 12, 24 and 208 weeks after therapy. Only patients who were evaluated in the long-term follow-up were included in the graphs.

MRI evaluation after 4 years showed an improvement in the fistula tracts in 67% [6/9] of MSC treated patients (Figure 2A). In only 4 patients (44% [4/9]) complete fibrotic fistula tracts were seen. The maximal fistula diameter found in all treated fistulas before bmMSC-therapy and 4 years thereafter was measured and showed significant improvement (6.1mm±1.2 vs. 2.6mm±0.9, P=.006) (Figure 2B). In none of the scans the presence of >2 cm collections was demonstrated, although in two patients smaller abscesses were observed. No *de novo* fistulas were found.

TABLE 2. Medication use and surgery. Medication use at the time of the 4-year follow-up visit and surgery during past 4 years in bmMSC treated patients. IFX=infliximab, ADA=adalimumab, VED=vedolizumab, MTX=methotrexate, I&D=incision and drainage.

	Cohort 1 (n=4)	**Cohort 2** (n=4)	**Cohort 3** (n=5)
Age at follow-up, mean, yr (min-max)	43 (31-57)	46 (43-51)	38 (26-52)
Male, n (%)	3 (75%)	4 (100%)	1 (20%)
Medication, n (%)			
No medication	1 (25%)	1 (25%)	2 (40%)
IFX / ADA	2 (50%)	2 (50%)	2 (40%)
VED	1 (25%)	1 (25%)	-
Thiopurines / MTX only	-	-	1 (20%)
Surgery, n (in number of patients) in last 4 years			
I&D	2 (1)	-	3 (3)
Ileocecal resection	-	1 (1)	-
Rectum extirpation	-	-	1 (1)
Setons in situ, number of patients (%)	1 (25%)	-	1 (20%)

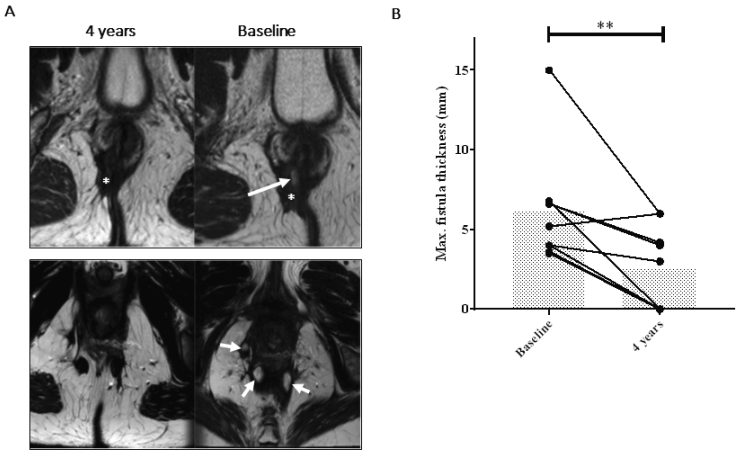

FIGURE 2. bmMSC-therapy causes fistula closure confirmed on pelvic MRI. A. Magnetic resonance images of the perianal region of two patients treated with bmMSC-therapy. Above: images at baseline and after 4 years from a patient treated in cohort 2. The fistula tract is completely closed, with only scar tissue being present 4 years after bmMSC-therapy. Under: images at baseline and after 4 years from a patient treated in cohort 3. Fistula tracts that contained fluid before bmMSC-therapy are closed 4 years later. Arrows - fluid inside the fistula tracts. Asterisks - scar tissue. B. Main fistula diameter (mm) at baseline and 4 years after MSC-therapy (n=9). ** p<0.01.

For each patient, the PDAI, CDAI, Vaizey score and quality of life measurements were compared between baseline and 4 years later. In 9 out of 13 patients, PDAI scores 4 years after bmMSC treatment were found to be lower than before bmMSC-therapy, which however did not reach statistical significance (4.3 vs. 3.8, P=.585) (Figure 3A). The CDAI revealed a significant lower disease activity 4 years after bmMSC-therapy (46.2 vs. 101.5 P=.014) (Figure 3B). No difference between the Vaizey score at baseline and after 4 years follow-up was found (Figure 3C). Comparison of the SF-36 and sIBDQ before and 4 years after bmMSC-therapy showed that bmMSC treated patients had improvement of their quality of life (sIBDQ; 54.8 vs. 60.1, P=.047, SF-36 MCS; 42.8 vs. 48.1, P=.089, SF-36 PCS; 52.2 vs. 52.8, P=NS) (Figure 3D and E).

Anti-HLA antibodies

In sera of patients included in the trial, we measured class I and II anti-human leukocyte antigen (HLA) antibodies, but none could be detected after 24 weeks (n=15) and 4 years of therapy (n=9).

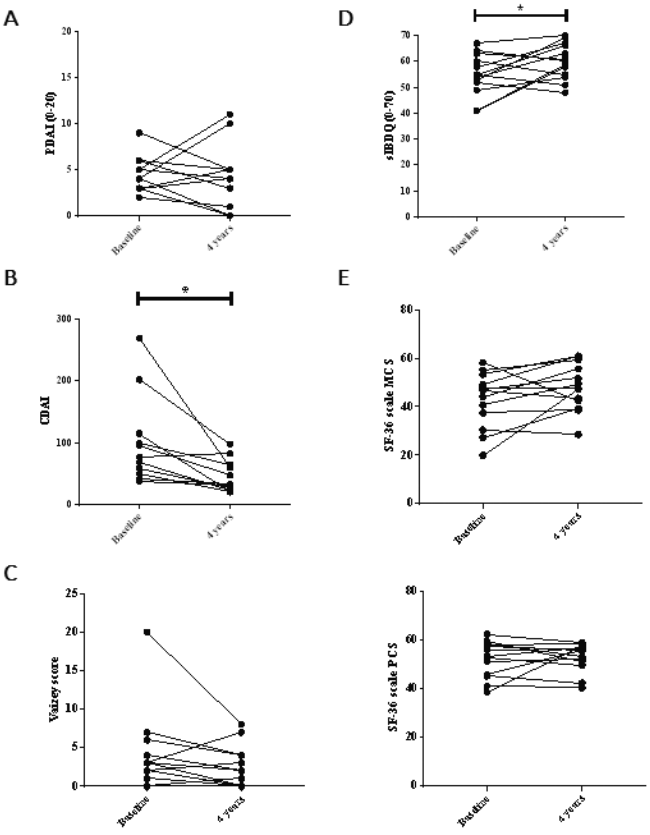

FIGURE 3. Improvement of quality of life after bmMSC-therapy. A. Perianal Disease Activity Index (PDAI) before and 4 years after bmMSC-therapy (n=13). B. Crohn's Disease Activity Index (CDAI) before and 4 years after bmMSC-therapy (n=11). C. Vaizey score for incontinence before and 4 years after bmMSC-therapy (n=11). D. Short Inflammatory Bowel Disease Questionnaire (sIBDQ) before and after bmMSC-therapy (n=13). E. Mental (MCS) and physical (PCS) component score of the Short Form (SF) 36 before and after bmMSC-therapy (n=13). *p<0.05.

TABLE 3. Clinical events in the past 4 years in bmMSC treated patients included in the long-term follow-up. Number of adverse events in total, (in number of patients).

	Cohort 1 (n=4)	**Cohort 2** (n=4)	**Cohort 3** (n=5)
Perianal abscess	2 (1)		4 (3)
Activity CD†	1 (1)	1 (1)	1 (1)
Infections‡	4 (2)	1 (1)	2 (2)
Gout	1 (1)		
Psoriasis guttae	1 (1)		
Uveitis		2 (1)	
Malignancy§		1 (1)	

†including diversion proctitis. ‡pneumonia, otitis media, fungal infection, periodontal abscess, laryngitis. §B-cell lymphoproliferative disease.

DISCUSSION

Perianal fistulas remain a common and challenging complication of CD with limited treatment options and invalidating complaints. In this follow-up study we evaluated the long-term safety and efficacy of bmMSC-therapy, as a new therapy for CD-associated perianal fistulas. bmMSC-therapy was already shown to be a safe and efficacious option for short-term closure of perianal fistulas in both our[9] and other clinical trials[10, 13-16]. The current study revealed that bmMSC-therapy is also safe and efficacious on the long-term.

The results after 24 weeks[9] showed that in cohort 1, 2 and 3 respectively 67%, 86% and 29% of the perianal fistulas were closed versus 33% in the placebo group. In this long-term follow-up we found fistula closure rates of 63%, 100% and 43% after 4 years in the 3 different MSC-cohorts. Therefore we concluded that fistula-healing persisted. In contrast, in the 3 placebo treated patients all fistulas were draining (again) after 4 years. Next to clinical evaluation of fistula closure, we found improvement of the fistula tracts in 67% of the bmMSC treated patients on pelvic MRI. However, complete fibrosis of fistula tracts was only observed in 44% of the patients. These results are in line with previous reports, showing that fistula tracks persisted on MRI despite (long-term) clinical remission[17]. Most of the patients evaluated in this long-term follow-up study experienced fistula closure already before 24 weeks of bmMSC treatment, but in two patients the fistula(s) closed only after 24 weeks (cohort 2 and 3). However, due to the low number of patients in our study and the loss of 2 patients in the long-term follow-up, caution is advised interpreting these results.

Besides high fistula closure rates in bmMSC treated patients, we also observed a significantly higher quality of life in bmMSC treated patients 4 years after therapy compared with their own baseline results. This endpoint is important since this directly reviews the effect of fistula closure on daily life. However, we also found a lower CDAI in bmMSC treated patients, suggesting that these patients had an overall lower CD activity 4 years later, which could also result in a better quality of life. In the future, it would also be interesting to take work-productivity and life style restriction into account when evaluating bmMSC-therapy for perianal fistulas.

Although so far no major safety concerns are raised in previous clinical trials using MSC-therapy[18], longer term safety aspects should always be evaluated carefully when using cell therapy. In this 4 years follow-up, 1 patient developed Epstein-Barr virus (EBV) positive B-cell proliferative disease in the rectum 4 years after treatment with EBV-negative MSCs, which is described in a case report[12]. In the end it was concluded that this LPD was not related to bmMSC-therapy, but more likely the result of prolonged immunosuppressive therapy since this patient used azathioprine, adalimumab and methotrexate in the past and currently vedolizumab. However, the possibility of additional local immunosuppression by bmMSC-therapy cannot be discarded completely. Furthermore, as already earlier described and judged not related to the bmMSC-therapy[9], one patient in our study died due to an adenocarcinoma in the caecum 2 years after treatment. Notwithstanding, their complete different origin and without any expected relation to MSC-therapy, these encountered malignancies warrant at least prudent (pre)-selection of patients and continuous long-term monitoring of local MSC-therapy.

To date, only a few papers evaluated the long-term outcome of patients treated locally with MSCs for refractory CD fistulas in terms of safety and efficacy. Ciccocioppo et al[19] showed that autologous bmMSCs were a safe therapy in 8 patients after 72 months of follow-up. The probability of fistula relapse-free survival was 88% after 1 year, 50% after 2 years and 37% after 4 years in this group of patients. Since it is still under debate whether autologous MSCs of IBD patients could be impaired like MSCs from systemic lupus erythematosus patients[20], disappointing long-term efficacy data could be related to the autologous origin of these cells. Other studies concerning local MSC-therapy for perianal fistulas only evaluated results up to 2 years[21-23]. In this regard, Panes et al[22] showed local Cx601, MSCs derived from adipose tissue, after 12 months to achieve a significantly higher proportion of fistula closure compared with controls (56% vs. 39%).

Interestingly, in our study none of the patients treated with bmMSC-therapy developed anti-HLA antibodies after 24 weeks or 4 years. This is in contrast to the treatment with

Cx601[10], in which in 34% (18 of 53) of the MSC treated patients found negative at baseline generated anti-HLA class I antibodies after MSC-therapy. The difference in the percentage of patients that formed anti-HLA antibodies might be explained by the origin of the MSC product, since Cx601 is a product consisting of MSCs derived from adipose tissue. The clinical relevance of the presence of anti-HLA antibodies is not elucidated yet, although no relation with response rates was observed in the Cx601 trial.

Of course, results of bmMSC-therapies from various clinical trials can only be properly compared when standardized and validated protocols are being used[24]. Our proposed protocol includes MRI and rectoscopy to localize and classify the tract(s) and closure of the internal orifice and curettage of the tract directly before bmMSC administration. Only patients without active luminal CD or strictures in the distal colon should be eligible.

In conclusion, we have shown that the efficacy of local bmMSC-therapy for perianal CD fistulas was maintained for up to 4 years after treatment. These long-term data show that bmMSC-therapy is not only able to heal refractory perianal fistulas in CD patients but also improve patients' quality of life. Although we have carefully judged the serious adverse events reported in this study and concluded that there was no relation with bmMSC treatment, more long-term safety data are needed from both clinical trials and daily clinical practice to fully appreciate all safety aspects concerning local bmMSC-therapy.

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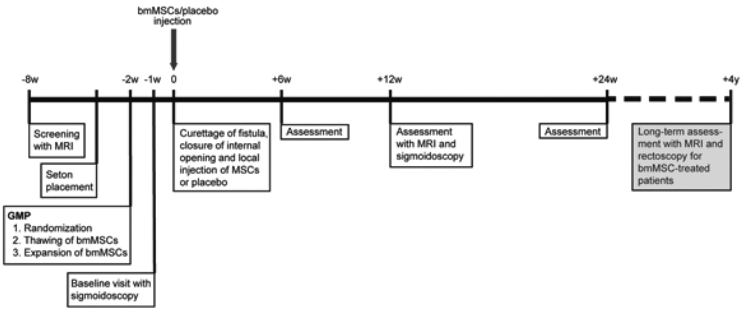
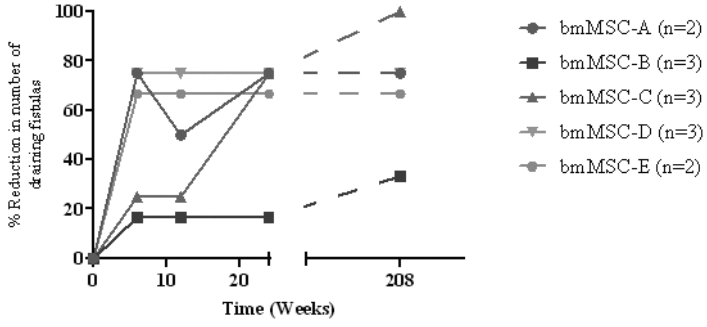
SUPPLEMENTARY FILES

Supplementary Table 1. Number of patients in long-term follow-up with closed fistulas per cohort and number of closed fistulas per cohort at 24 weeks and 4 years.

	Cohort 1	Cohort 2	Cohort 3
Patients with closed fistulas at 4yr, n (%)	3/4 (75%)	4/4 (100%)	1/5 (20%)
Patients with closed fistulas at 24wk, n (%)	3/4 (75%)	3/4 (75%)	1/5 (20%)
Closed fistulas at 4yr, n (%)	5/8 (62.5%)	6/6 (100%)	3/7 (42.9%)
Closed fistulas at 24wk, n (%)	5/8 (62.5%)	5/6 (83.3%)	2/7 (28.6%)



Supplementary FIGURE 1. Short- and long-term follow up of patients treated in the randomized placebo-controlled dose-finding trial 'Allogeneic Bone Marrow Derived Mesenchymal Stem Cells for the Treatment of Fistulas in Patients with Refractory Perianal Crohn's Disease' [NCT01144962].



Supplementary FIGURE 2. Fistula closure in relation to bmMSCs from different donors.