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Adipose mesenchymal stromal cell-based therapy for severe osteoarthritis of the knee: A phase I dose escalation trial.

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CONFLICT OF INTEREST

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KEY WORDS

Osteoarthritis, adipose mesenchymal stromal cells, intra-articular injection, therapeutic potential, regenerative medicine, phase I clinical trial

ABSTRACT

Objectives. Osteoarthritis (OA) is the most widespread musculoskeletal disorder in adults leading to cartilage damage associated with subchondral bone changes and synovial inflammation, and causing pain and disability. The present study aimed at evaluating the safety of a dose-escalation protocol of intra-articular delivered adipose-derived stromal cells (ASCs) in patients with knee OA, as well as clinical efficacy as secondary endpoint.

Methods. A bicentric, uncontrolled, open phase I clinical trial was conducted in France and Germany upon regulatory agencies approval for ASC expansion procedure in both countries. Eighteen consecutive patients with symptomatic and severe knee OA have been treated with a single intra-articular (IA) injection of autologous ASCs between April 2012 and December 2013. The study design consisted of three consecutive cohorts (6 patients each) with a dose-escalation: low-dose (2×10^6 cells), medium-dose (10×10^6), and high-dose (50×10^6). The primary outcome parameter was safety evaluated by recording adverse events (AEs) throughout the trial and secondary parameters were pain and function subscales of the WOMAC.

Results. After 6 months of follow-up, the procedure was safe and no serious AEs were reported. Four patients experienced transient knee joint pain and swelling after local injection. Interestingly, patients treated with the low-dose of ASCs significantly improved in pain and function compared to baseline.

Conclusion. Our data suggest that the intra-articular injection of ASCs is a safe therapeutic alternative to treat severe knee OA patients. A placebo-controlled double-blind phase IIb study is being initiated to assess clinical and structural efficacy.

Trial registration number: NCT01585857

INTRODUCTION

Osteoarthritis (OA) is a multifactorial, slowly progressive degenerative disorder of the joints leading to the irreversible damage of the cartilage, sclerosis of subchondral bone and synovial inflammation [1]. As a consequence of the increasing longevity and obesity, the burden cost of OA to the healthcare system rapidly grows. The current treatment strategies have no impact on the progressive degeneration of joint tissues. In this context, use of mesenchymal stromal stem cells (MSCs) is an attractive therapeutic option thanks to their chondrogenic and anti-inflammatory properties [2]. Adipose tissue-derived MSCs (ASCs) share similar properties with bone marrow-derived MSCs but are easier to collect for clinical application with higher isolation yields. Indeed, intra-articular injection of ASCs prevented OA onset in a collagenase-induced murine knee OA model and reduced synovitis, osteophyte formation and cartilage degeneration [3]. Furthermore, intra-articular injection of 2 or 6 millions of autologous ASCs improved the cartilage degradation score and significantly reduced knee synovitis in a biomechanical induced OA rabbit model [4].

Using an established GMP procedure based on ASCs expanded for 2 weeks in the presence of platelet lysate [5], we conducted a proof-of concept phase I clinical trial to assess the safety and efficacy of intra-articular injection of autologous ASCs in patients with active and severe knee OA.

PATIENTS AND METHODS

Study design

A phase I, prospective, bi-centric, single-arm, open-label, dose-escalating clinical trial of a single injection of autologous ASCs in patients with severe primary knee OA was conducted between March 2012 and April 2014 in two hospitals: CHRU Montpellier (France) and the Department of Orthopaedic Surgery at the University of Würzburg (Germany). No placebo group was scheduled due to ethical issue to include late stage knee OA patients associated with a liposuction without active therapy benefit. The study protocol was approved by the local ethic committees of both institutions (Comité de Protection des Personnes of Montpellier (ref UF8606-120203) and Ethik-Kommission bei der Medizinischen of Würzburg) and by the national competent authorities (ref TC301; EudraCT N°: 2011-000183-10).

Patient selection and enrolment

A total of 48 outpatients with knee OA were screened (Figure 1). Eighteen consecutive patients with primary femorotibial knee OA diagnosed according to the clinical and radiological criteria of the American College of Rheumatology were enrolled in this study after written informed consent was obtained [6].

Inclusion criteria

Patients between 50 to 75 years of age, with symptomatic primary knee OA and radiographic changes of grade 3 to 4 according to the Kellgren-Lawrence (K/L) scale in the targeted knee were included [7]. In order to get histological analysis for safety issues, the medical board required end stage knee OA patients with an indication of knee prosthesis in the year

following the inclusion. Symptomatic primary knee OA was defined by a daily knee pain for at least 12 months before study inclusion.

Exclusion criteria

Patients were excluded if they had secondary arthritis (related to rheumatoid arthritis, spondyloarthritis, previous articular fractures, post-infectious arthritis and crystal arthropathies), autoimmune disorders, and previous malignancies in the past 5 years. Previous administration of oral/intra-articular corticosteroids and injection of hyaluronic acid derivatives within 6 months before screening examination were also exclusion criteria.

Treatment allocation

Eligible patients were consecutively allocated to the treatment groups, three arms with different doses (2×10^6 cells, 10×10^6 cells and 50×10^6 cells) (Figure 1). The starting dose of $2x10^6$ cells has been defined based on the No Observed Adverse Effect Level (NOAEL) obtained after IA administration determined in preclinical studies performed in the goat and the rabbit models of OA, adjusted by allometric factors (weight and size of the knee joint compared to human) ([4] and data not shown).

First, the patients underwent an outpatient liposuction in France or Germany under local anesthesia and the autologous ASCs were produced and prepared on a single GMP-facility (Etablissement Français du Sang Midi-Pyrénnées, France), as summarized in the supplementary section. Fourteen days after isolation, ASCs were recovered and underwent a defined quality control prior to shipping (see supplementary text). A single intra-articular dose of ASCs was injected into the knee joint (volume 5mL) under ultrasound control.

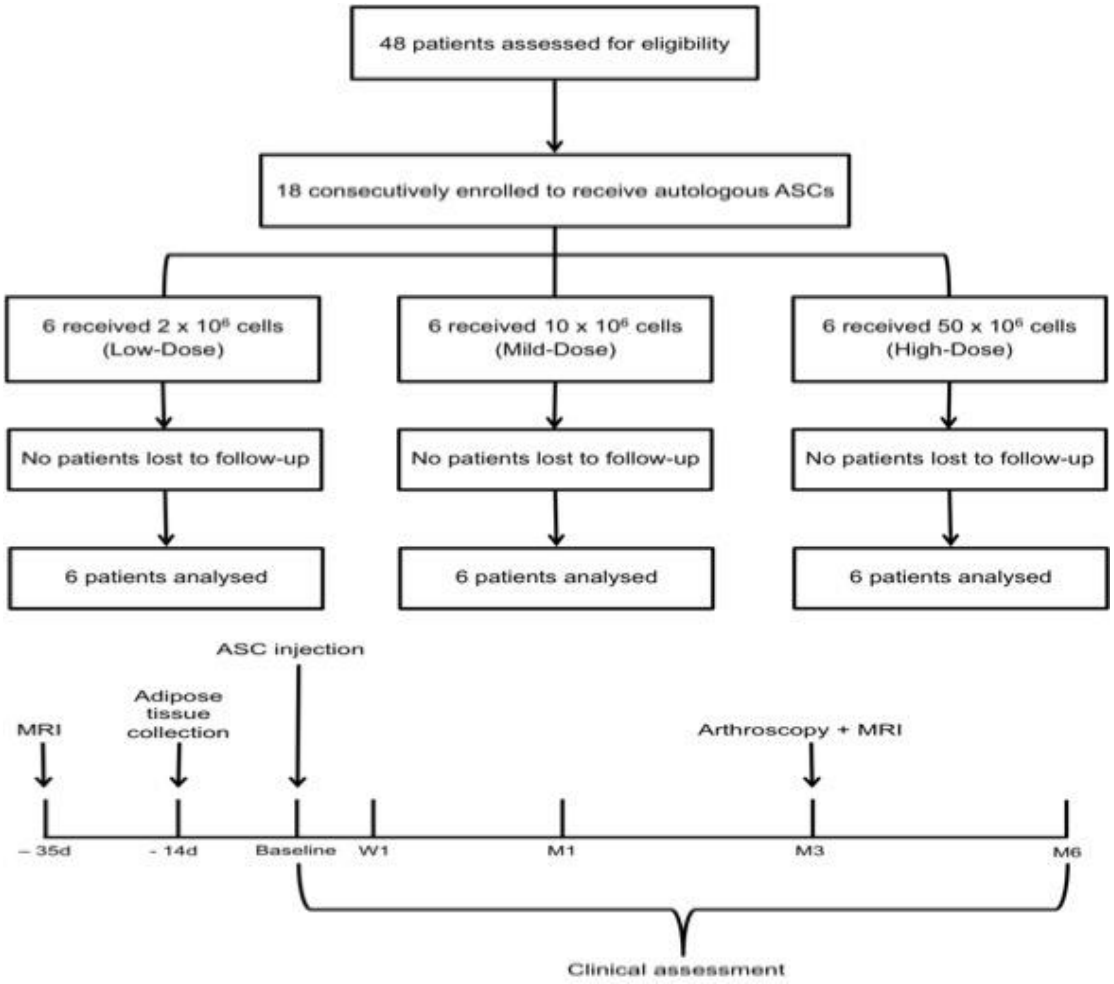

Fig. 1

Cell preparation and expansion of ASCs

The procedure was already described elsewhere [5]. The stromal vascular fraction (SVF) was obtained by means of collagenase digestion. Aliquots of 10 g of adipose tissue were mixed with 34 mL of the collagenase solution (NB6; Coger, Paris, France) and incubated at 37°C for 45 min. Enzymatic digestion was stopped by the addition of complete culture medium (CCM) containing minimum essential medium (MacoPharma, Tourcoing, France), human platelet growth factor enriched plasma, 10 mg/mL ciprofloxacin and 1 U/mL heparin. After homogenization, the digested suspension was passed through sterile 100-mm filters. The cells were centrifuged at room temperature for 10 min at 600g. The supernatant was discarded and

the SVF was resuspended in 20 mL of CCM. An aliquot of the SVF was removed for the quality controls: cell count, viability, phenotyping (CD34, CD45 and CD14) and sterility. The cells from the SVF were then seeded in a 1270-cm^2 CellStack culture chamber (MacoPharma) at a density of 4.10^3 cells/cm^2 in CCM, with the use of seeding kit (MacoPharma), at 37°C in an atmosphere saturated with moisture and 5% CO_2. After an initial 24-h incubation, the non-adherent cells were removed. The adherent cells were washed once with Dulbecco' s phosphate-buffered saline (PBS), and CCM medium was added for 7 days. The medium was completely replaced at day 4 and day 6 of culture with the use of medium exchange kits (MacoPharma). At day 8 (primary culture, P0), the cells were harvested with the use of a detachment kit (MacoPharma) according to the following protocol: after aspiration of the medium and washing with Dulbecco' s PBS, 50 mL of irradiated trypsin solution was added for 5 min at room temperature. After the inhibition of trypsin activity by the addition of CCM, the cells were collected in a transfer bag (MacoPharma). An aliquot of the cell suspension was aseptically removed for cell count, viability, phenotyping (CD34, CD45 and CD14), measures of hTERT messenger RNA (mRNA) contents by qRT-PCR and assessment of microbial testing.

The cells were seeded in a 1270-cm^2 Cell-Stack culture chambers at a density of 2×10^3 cells/cm^2 and incubated for 6 days. The CCM was completely replaced at day 11 and day 13. At day 11, an aliquot of culture medium was aseptically removed for mycoplasma and endotoxin testing. At day 14, the cells were harvested according to the same procedure as described above. The cell suspension was placed in a transfer bag (MacoPharma) and washed with Dulbecco's PBS. The ASCs were then resuspended in a solution containing 3.6% human albumin provided by Laboratoire français du Fractionnement et des Biotechnologies (Courtaboeuf, France) and a poly-ionic solution containing glucose. An aliquot of the ASC

suspension was aseptically removed for cell count, and its quality was evaluated as described above.

Flow cytometry analyses were performed as described below. Briefly, ASCs (2×10^5 cells) were stained with saturating amounts of monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) and their respective isotype controls for 30 min in the dark at 4° C in PBS/0.5% human albumin and 0.1% sodium azide. After washing, the labelled cells were analyzed by flow cytometry (EPICS XL-MCL flow cytometer; Beckman-Coulter, Nyon, Switzerland). FITC anti-CD14, FITC anti-CD45, PE anti-CD34, PE anti-CD73, PE anti-CD90, PE anti-CD105 and immunoglobulin (Ig) G1 PE and FITC were from BD Pharmingen (Le Pont de Claix, France).

Release criteria of ASCs

Release criteria were defined as negative for microbial testing on SVF, intermediate product (P0) and final product (P1), negative for mycoplasma testing on adipose tissue and culture medium at day 11, endotoxin testing negative on culture medium at day 11, absence of hTERT detection by qRT-PCR on intermediate product (P0). Finally, on active substance, cellular viability had to be > 90%. The percentage of positive cells for haematopoietic markers (CD45 and CD14) had to be lower than 2% and for mesenchymal markers had to be higher than 90% for CD90 and CD73 and more than 80% for CD105. The percentage of positive cells for CD34 marker had to be less than 10%. Karyotypes analyses have been performed, on final product, for 15 productions. Due to the time required for performing the karyotype, results have been obtained after the release. Karyotypes analyses revealed no clonal abnormalities. Results for release criteria obtained for the 3 cohorts are presented in the supplementary section.

Outcome measures

Primary endpoint

Incidence, relatedness and severity of treatment-emergent suspected unexpected serious adverse reactions (SUSARs), serious adverse events (SAEs), adverse events (AEs) were documented at each visit throughout the study. Laboratory tests (hematology, blood chemistry, urinalysis), vital signs and physical examinations of the patients were assessed systematically. A 12 weeks safety period was implemented between subject 1 and subject 2 of the first cohort receiving the low dose and the safety medical board authorized to continue with patients 2 to 6. A further 4 weeks safety period was scheduled between the other two cohorts.

Secondary endpoints

The secondary efficacy endpoints were assessed by measuring WOMAC (Western Ontario and McMaster universities osteoarthritis), pain VAS (Visual Analogic Scale), the PGA (Patient Global Assessment), the SAS (Short Arthritis Assessment Scale) and KOOS index (Knee injury and Osteoarthritis Outcome Score) [8]. A 0-100 mm VAS was used to assess WOMAC pain (five questions), physical function (17 questions), and stiffness (two questions) subscales. OARSI (OsteoArthritis Research Society International)/OMERACT (Outcome Measures in Rheumatology) response was defined as 20% improvement compared to baseline VAS and WOMAC index [9] . Quality of life was measured by the short-form 36 (SF-36) questionnaire [10].

Secondary imaging endpoints included dGEMRIC and T_{1rho} MRI for selected German patients at 3-4 months after the ASC injection [18]. MRIs were evaluated by a radiologist blindly of the administered dose. dGEMRIC and T_{1rho} maps were motion corrected, zero-filled and then derived using anatomical landmarks and an automated fit algorithm [11].

Histology

Upon request of the ethical committee, a total knee arthroplasty (TKA) was originally scheduled three months after ASC injection for all the patients in order to obtain histological analysis. However, if the patient refused TKA, a knee arthroscopy with biopsy could be performed. No standardized protocol was planned for biopsies. Cartilage and synovial samples were fixed 24h in 10% neutral formol and embedded in paraffin. Sections of 5µm thickness were stained with Hematoxylin-Eosin, Alcian Blue or Toluidine blue. Immunohistochemistry was performed on a Benchmark Ultra Ventana® automat with the following antibodies: protein S100 (DAKO®, 1/3200, polyclonal) CD34 (DAKO®, 1/100, QBEND/10) and Ki67 (DAKO®, 1/100, monoclonal mouse, clone Mib-1). The OARSI cartilage OA histopathology grading system was performed blindly by an experienced anatomopathologist [12].

Statistical analysis

All values are expressed as mean +/- standard deviation. The significance of differences was assessed either by Wilcoxon test or by one-way analysis of variance (ANOVA) and the corresponding nonparametric tests. A value of $p < 0.05$ was considered statistically significant. All analyses were performed using GraphPad Prism software version 6.0 (GraphPad Software, La Jolla, CA).

RESULTS

Characteristics of patients

All 3 cohorts revealed similar baseline characteristics for age, sex, and body mass index. 83% of patients were grade IV in the K-L scale (Table 1). Finally, 11 patients were included in France and 7 in Germany. Baseline levels for pain and function (WOMAC, KOOS, SAS scores) were different between the cohorts (Table 1). The disease activity at baseline was higher in the group of patients injected with the low dose of ASCs, with higher VAS and WOMAC values. All patients completed the 6-months follow-up. Only one patient with a persistent joint swelling and knee pain underwent TKA surgery at 6 month.

Safety and tolerance profile of autologous ASCs intra-articular injection

No adverse effect (AE) associated with liposuction and IA injection was observed in this study (Table 2). No serious infectious AEs occurred during the follow-up related to ASC injection (Table 2). Laboratory tests, vital signs, and electrocardiograms indicated no local or systemic safety concerns.

One severe adverse effect (SAE), an unstable angina pectoris without increased cardiac markers, was reported in one patient, 3 months after ASC injection. The patient's risk factors included hypertension and hyperlipidaemia. Five minor AEs reported by 4 patients were potentially related to the procedure: slight knee pain/joint effusion occurred during the first week after ASC injection, which resolved with non-steroidal anti-inflammatory drug (NSAID) in 3 patients and spontaneously in 1 patient without medication (Table 2). Otherwise, a small increase for creatinine-phosphokinase (CPK) and alanine aminotransferase (ALT) was observed in two and one patient, respectively. We also reported a mild decrease of neutrophil count in one patient who presented a low baseline count (1500/mm^3) and high variability of the neutrophil count, independently of IA injection, during the follow-up.

Efficacy profile of autologous ASC injection on OA clinical outcomes

Mean changes from baseline to one week, 3 months and 6 months in clinical outcomes are summarized in table 3. An improvement for all the clinical outcome parameters (pain, function, mobility) regardless of the injected dose was observed (Figure 2). However, statistical significance was detected only for patients treated with the low dose. Finally, all patients except one denied having the previously scheduled TKA.

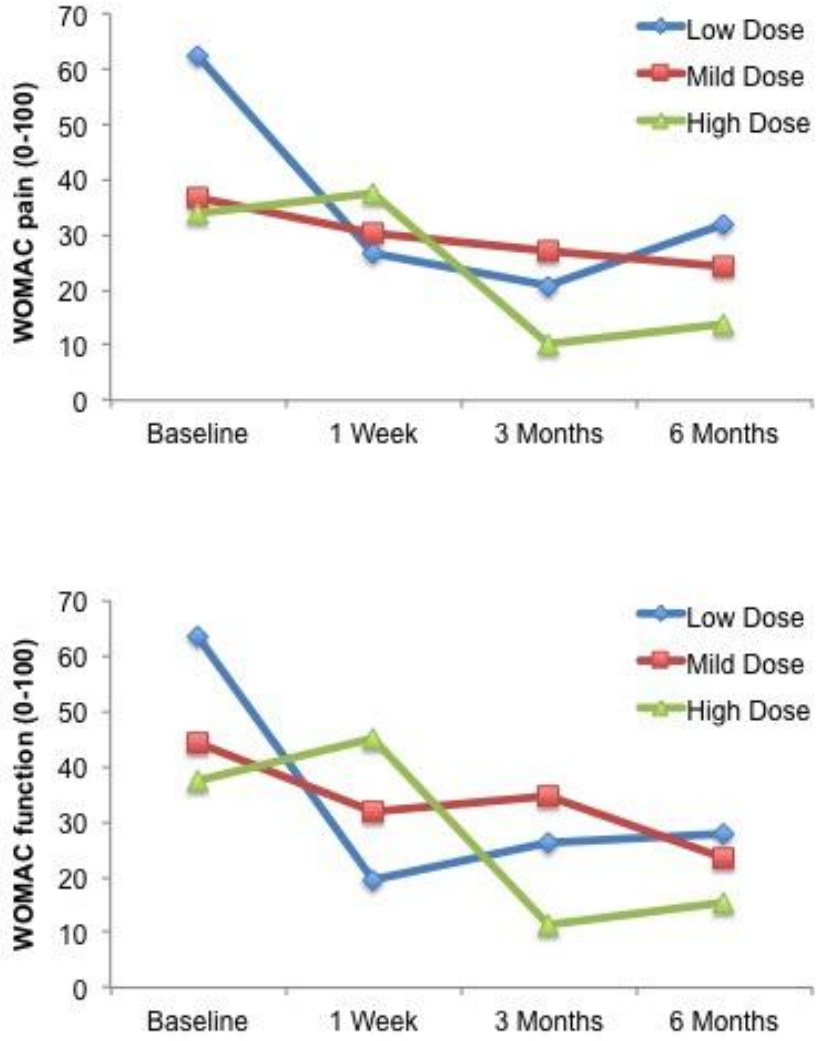

Fig. 2

MRI Evaluation

Among the 7 patients included in Germany, quantitative dGEMRIC (6 patients) and T_{1rho} (5 patients) maps were acquired and analyzed prior to and 4 months after therapy (Figure 3). In these parameter maps the dGEMRIC index increased in 3 selected patients with time, while the T_{1rho} values decreased at the same time. For the other 3 patients the opposite effect was observed. Thus, the positive changes were only limited and suggested a possible cartilage improvement in 3 out of 6 patients. In conclusion, within this small number of patients, we did not observe any correlation between MRI and clinical changes.

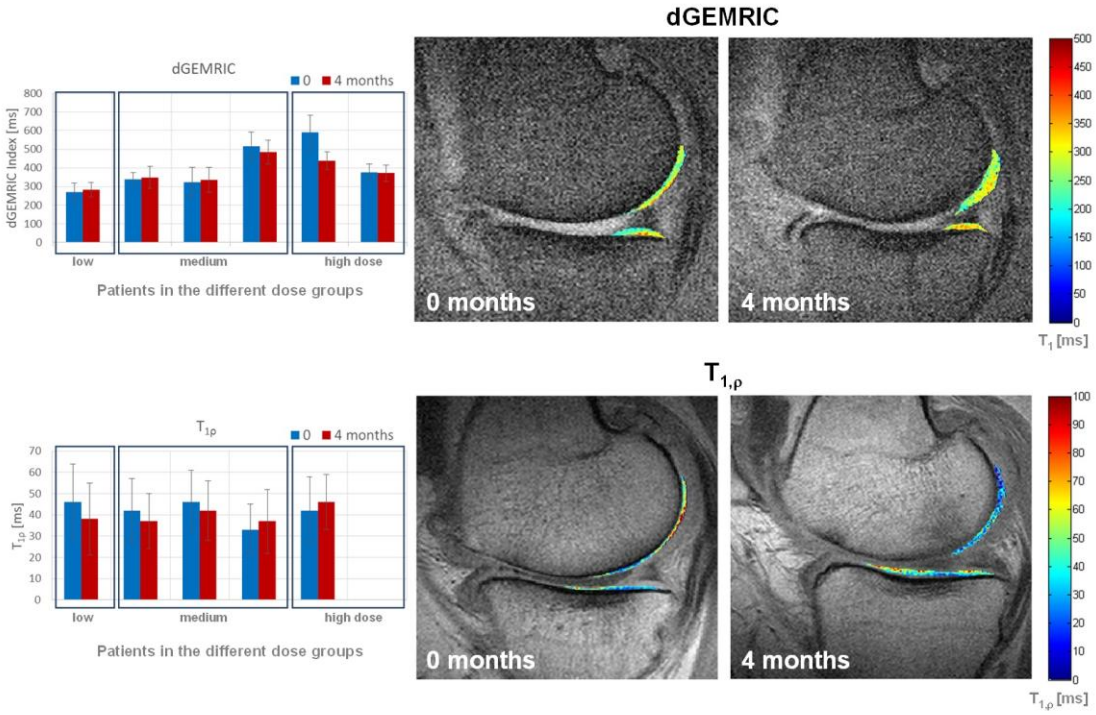

Fig. 3

Histological analysis

Histological analysis of cartilage and synovium was available for 11/18 patients after arthroscopy after 3 months. All samples showed signs of severe OA (OARSI histological grading >3). Osteoarthritic chondrocytes stained positive for PS100 and negative for CD34 or

Ki67 (Figure 4). Significant synovial inflammation was absent in 2 cases while weak or moderate inflammation and synovial hyperplasia with diffuse interstitial lymphocytic infiltrate were observed in 5 and 4 cases, respectively. In one patient (case 2) who received a low dose of ASCs, we observed sheet of cells that could be interpreted as stem cell graft on cartilage surface (Figure 4). These cells showed rare Ki67 nuclear staining, weak PS100 staining and were CD34 negative. Finally, none of the synovial or cartilage samples showed any tumor proliferation.

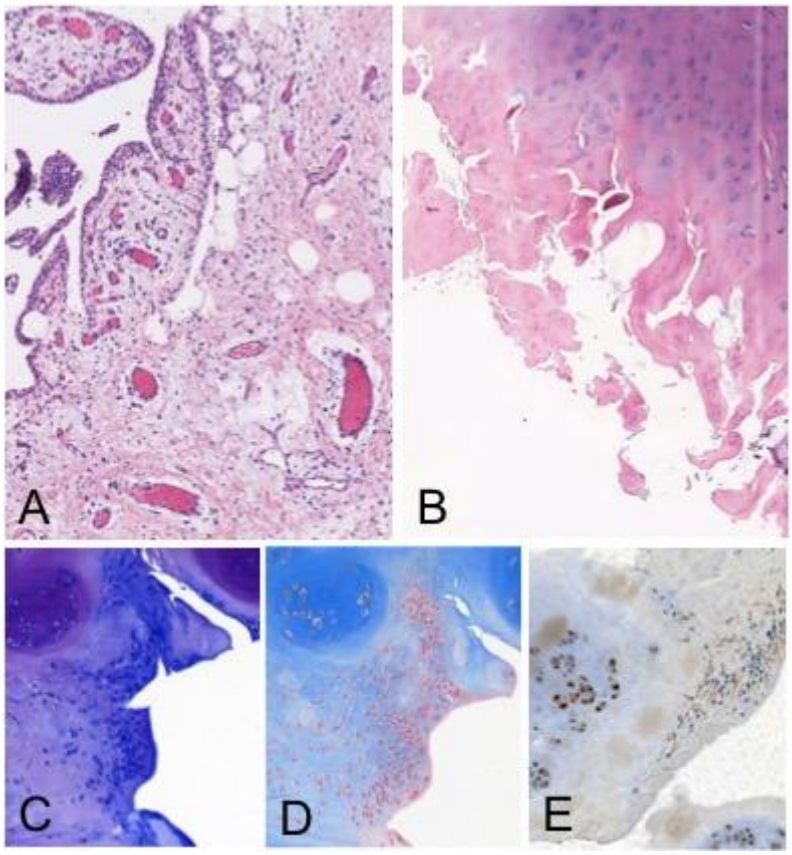

Fig. 4

DISCUSSION

This pilot trial reached its predetermined primary outcome parameters, i.e. safety of intra-articular injection of ASCs in patients with knee OA. Our results are similar to those reported from other studies, in critical limb ischemia or fistulae in inflammatory bowel disease where ASCs have been injected locally without reported side effects [5, 13]. Additionally, we report clinical improvement with a reduction in pain levels and WOMAC score in all 3 groups, even though statistically significant results were only obtained in the low dose group. Actually, the large variability in the range of the initial clinical parameters as well as the limited sample size may explain why statistical significance was not reached at 6 months. However, when compared to historical control studies, our approach seems very encouraging. For example, in a thoroughly double-blind study on hyaluronic acid treatment into the knee, the WOMAC pain score decreased by 22.9±1.4 mm between baseline and 6 months [14]. In the present study, WOMAC pain score decreased by 30.7±10.7 mm in the group receiving low doses of ASCs. Furthermore, the average difference from baseline to 6 months on the WOMAC subscale scores (pain, function and stiffness) is higher than the recommended minimal perceptible clinical improvement of 10 mm [15]. Additionally, a study comparing hyaluronic acid with saline solution reported 54.6% OARSI responders in the saline group after 13 weeks [16]. This score is lower than the OARSI response obtained with the 3 different doses at the same time-point in the present study with 83.3% in low dose, 60% in medium dose and 60% in high dose group. In a recent controlled study with steroid as comparator, the magnitude of the placebo effect led to a decrease in WOMAC pain score of 20 mm at 6 months versus baseline [17]. They recorded 52.1% OARSI responders at 6 months, which is lower than obtained in our different groups. These studies suggest that ASC therapy might be more efficient than a possible placebo effect.

Our results are also consistent with those obtained in a recent study in patients with a larger heterogeneity in age and less severe forms of OA [18]. In a recent similar study from Jo et al., the highest efficiency was found at the highest dose (100×10^6 cells) in patients who presented the highest levels of pain at baseline (VAS and WOMAC) [19]. In our study, the group of patients injected with 2×10^6 cells exhibited the best response to ASC treatment while they had higher baseline pain and WOMAC scores compared to higher doses. One possible reason for this inverse dose-effect of ASC therapy might be explained by the higher level of inflammation in the lowest dose group, as reflected by the highest level of pain at baseline. The inflammatory milieu might have primed the injected ASC to exert their immunomodulatory functions more efficiently than in the groups where the inflammation was lower. We therefore cannot rule out that the treatment response was partly dependent to the initial disease activity. Orozco et al. published another interesting study on the treatment of knee OA with autologous MSCs derived from bone marrow [20]. They injected 40×10^6 cells into the knee joint. Improvement of cartilage morphology and quality was observed in almost all patients using MRI T_2 mapping, suggesting a possible structural benefit of stem cell therapy.

The potential mode of action of ASCs for the treatment of OA includes at least 3 different biological effects. The first is direct differentiation of ASCs into chondrocytes, while the others are related to a possible paracrine effect of secreted bioactive molecules, including anti-inflammatory and chondroprotective mediators. However, the capacity of MSCs to differentiate into chondrocytes is probably not critical in the observed therapeutic effect. Preliminary studies in rabbit and goat have shown that cartilage regeneration did not occur at the expense of chondrogenic differentiation of the injected cells but may be strongly related to a secondary stimulation of endogenous progenitor cells through paracrine effects [21]. MSCs

contributed to the repair of damaged articular cartilage through homing, engraftment, production of cartilage matrix and reduction of local inflammation [22-25]. Stromal cells have been shown to possess immunomodulatory and anti-fibrotic properties, to protect cells from oxidative stress and apoptosis and, to stimulate proliferation and chondrogenic differentiation in co-culture through secretion of growth factors [23]. In pre-clinical models of OA or experimental models of inflammatory diseases such as arthritis and experimental encephalitis, the benefit of ASC injection was related to secretion of anti-inflammatory factors including HGF, HLA-G5 or IL1RA [26]. The immunomodulatory properties of adipose-derived MSCs are even stronger than those from other tissue sources (for review, see [27]). Whether the *in vitro* capabilities of MSCs from different tissue sources reflect the *in vivo* situation has still to be elucidated. Nevertheless, there is an obvious variation among donors that could be related to differences between isolation, expansion and freezing/thawing procedures. All together, these data suggest that MSCs can reduce synovitis and favor an appropriate environment for tissue regeneration through expression of active growth factors, or by recruitment of endogenous progenitors.

To conclude, although this phase I study included a limited number of patients without a placebo arm, we were able to show that this innovative treatment was safe and well tolerated in patients with knee OA. We also provided encouraging preliminary evidence of efficacy. Larger and controlled long-term studies are now mandatory to confirm whether this new strategy of cell therapy can improve pain and induce structural benefit. Moreover, it is likely that similar therapeutic procedure based on autologous ASCs can be extended in the future to other joints, such as the hip joint or indications such as intervertebral disc degeneration.

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CONFLICT OF INTEREST

All the authors declared no competing interests.

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FIGURE LEGENDS

Figure 1: Flow chart of the clinical trial

Figure 2: WOMAC pain and function improvement during the study

Figure 3: Magnetic Resonance Imaging (MRI)

dGEMRIC and T_{1rho} MRI of selected patients. The graphs on the left show the dGEMRIC and T_{1rho} values of 6 (5) patients prior to and 4 months after cell therapy. Increasing dGEmRIC and decreasing T_{1rho} values are each known to correspond to increasing GAG/PG content and thus improved cartilage condition. On the right, the corresponding dGEMRIC and T_{1rho} maps are shown as a colour coded overlay on an anatomical MR image for the patient receiving a low cell dose. The observed values in the cartilage change in the time course as can be easily seen and correspond to an increase in cartilage condition.

Figure 4: Histological findings

A: Vascular congestion and weak lymphocytic infiltrate of the synovial (case 8)

B: Osteoarthritic cartilage OARSI grade >3 (case 4)

C: Toluidine Blue staining (case 2)

D: Stem cell stroma shows an Alcian Blue depleted matrix compared to the strong staining of osteoarthritic cartilage (case 2)

E: Weak PS100 staining of possible stem cells on the cartilage surface and strong PS100 staining of chondrocytes (case 2)

TABLES

Table 1: Patient demographic and baseline characteristics between each group (low-, medium-, and high-dose).

	Low-dose (n=6)	**Medium-dose (n=6)**	**High-dose (n=6)**
Age (years)	63.2 ± 4.1	65.5 ± 8.1	65.2 ± 2.3
Women, n (%)	3 (50)	3 (50)	4 (66.7)
BMI (kg/m^2)	28.8 ± 1.5	26.96 ± 3.1	27.1 ± 2.4
Kellgren and Lawrence, n (%)			
Grade III	2 (33)	1 (17)	0
Grade IV	4 (66)	5 (83)	6 (100)
WOMAC index scores (0-100 scale)			
Pain subscale	62.5 ± 15.5	36.6 ± 14.6	34.0 ± 25.6
Stiffness subscale	58.5 ± 27.9	54.5 ± 17.9	45.3 ± 31.5
Function subscale	63.6 ± 16.7	44.4 ± 17.9	37.3 ± 26.5
Total index	60.7 ± 18.6	47.2 ± 14.7	38.8 ± 27.3
Global knee pain VAS (0-100 mm)	77 ± 15.7	63.7 ± 20.5	43.7 ± 25.4
PGA (0-100 mm)	30 ± 21	32 ± 17.9	46.7 ± 20.7
KOOS index (0-100 mm)	34 ± 15	42 ± 9	45.2 ± 13.6
SAS index (0-40 mm)	29 ± 6	26 ± 5	19.5 ± 7.6
SF-36			
Physical scale	30.9 ± 8.2	29.9 ± 6.2	35.7 ± 10.6
Mental scale	55.9 ± 8.3	51.9 ± 10.2	53.6 ± 7.8

Data are mean ± SD or percentage.
N: number; BMI: body mass index; WOMAC: Western Ontario and McMaster Universities Osteoarthritis index; VAS: visual analogue scale; PGA: patient global assessment; KOOS: Knee injury and Osteoarthritis Outcome; SAS: short arthritis assessment scale; SF-36: short-form 36 (quality of life).

Table 2: Summary of adverse events during the clinical trial

	0-3 Months			Months 3-6		
Variables	**Low-Dose (n=6)**	**Medium-Dose (n=6)**	**High-Dose (n=6)**	**Low-Dose (n=6)**	**Medium-Dose (n=6)**	**High-Dose (n=6)**
Adverse events; n.	10	4	13	8	2	1
Patients with AEs; n (%)	6 (100)	4 (67)	5 (83)	6 (100)	2 (33)	3 (50)
Patients with serious AEs; n.	0	1	0	0	0	0
Patients with serious infectious events; n.	0	0		0	0	0
Biological changes[*]						
CRP > 1-3ULN; no	2			1		
ALT > 1-3 ULN; no		1				
CPK > 1-3 ULN; no				1		1
Mild Neutropenia, 900-1499 cells/mm^3	1			1		
Infections						
Nasal Congestion	1	1				
Rhinitis and pharyngitis			3			
Influenza syndrome		1				
Urinary infection	1					
Dental Infection	1					
Musculoskeletal disorders						
Joint effusion/swelling treated knee[†]	1		3	1		
Sciatic pain				1		
Low back pain			2			
Trauma to the treated knee			1		2	
Skin erythema around the treated knee				1		
Shoulder pain	2					
Hip pain	1					
Neurological disorders						
Headache			1			
Gastrointestinal disorders						
Diarrhoea			1			
Eye disorders						
Cataract				2		
Conjunctivitis			1			
Cardiovascular disorders						
Right coronary artery stenosis[‡]		1				

AEs: adverse events; n: number; CRP: C-reactive protein; ALT: Alanine Aminotransferase; CPK: Creatinine Phospho Kinase

[*]The incidence is shown for participants who had normal values at baseline

[†] 5 adverse events related to ASCs

[‡] 1 serious adverse events not related to ASCs

Table 3: Effect of autologous ASC injection on OA clinical outcomes.

2×10^6 injected cells	**Δ 1 week**	**p value**	**Δ 3 months**	**p value**	**Δ 6 months**	**p value**
WOMAC pain	- 36.0 ± 10.2	<0.001	- 41.7 ± 10.2	<0.01	- 30.7 ± 10.7	<0.05
WOMAC stiffness	- 41.2 ± 10.6	<0.01	- 46.8 ± 10.6	<0.001	- 35.3 ± 11.1	<0.05
WOMAC function	- 44.0 ± 12.2	<0.01	- 37.4 ± 9.9	<0.01	- 35.7 ± 10.5	<0.01
WOMAC total	- 38.6 ± 8.6	<0.001	- 41.2 ± 8.5	<0.001	- 33.1 ± 8.9	<0.001
VAS pain	- 51.5 ± 12.7	<0.01	- 54.4 ± 12.7	<0.01	- 41.2 ± 13.3	<0.05
KOOS index	+ 34.9 ± 8.7	<0.01	+ 38.0 ± 8.7	<0.001	+ 31.8 ± 9.1	<0.01
SAS index	- 15.2 ± 4.8	<0.05	- 16.3 ± 4.8	<0.01	- 11.3 ± 5.0	0.09
OARSI/OMERACT responders, %	ND		83.3		80.0	
SF-36						
Physical scale	+ 6.6 ± 4.4	0.34	+ 12.8 ± 4.4	<0.05	+ 8.2 ± 4.6	0.33
Mental scale	- 52.9 ± 3.0	0.75	- 0.9 ± 3.7	0.99	- 4.0 ± 3.8	0.60
10×10^6 injected cells	**Δ 1 week**	**p value**	**Δ 3 months**	**p value**	**Δ 6 months**	**p value**
WOMAC pain	- 6.3 ± 9.5	0.85	- 9.7 ± 9.9	0.65	- 12.4 ± 9.9	0.47
WOMAC stiffness	- 27.7 ± 10.9	0.052	- 16.2 ± 11.4	0.37	- 30.1 ± 11.4	<0.05
WOMAC function	- 12.7 ± 10.7	0.51	- 9.9 ± 11.2	0.71	- 20.9 ± 11.2	0.19
WOMAC total	- 19.7 ± 9.1	0.11	- 12.7 ± 9.6	0.43	- 22.9 ± 9.1	0.054
VAS pain	- 20.8 ± 11.9	0.22	- 22.2 ± 11.9	0.18	- 27.0 ± 11.9	0.09
KOOS index	+ 5.9 ± 6.5	0.69	+ 4.9 ± 6.5	0.79	+17.2 ± 6.5	<0.05
SAS index	- 4.8 ± 3.6	0.41	- 4.8 ± 3.6	0.41	- 11.7 ± 3.6	<0.05
OARSI/OMERACT responders, %	ND		60.0		60.0	
SF-36						
Physical scale	- 0.62 (4.0)	0.99	+ 2.1 (4.0)	0.92	+ 5.4 ± 4.0	0.42
Mental scale	+ 4.7 (5.8)	0.76	+ 0.1 (5.8)	0.99	+ 3.2 ± 5.8	0.91
50×10^6 injected cells	**Δ 1 week**	**p value**	**Δ 3 months**	**p value**	**Δ 6 months**	**p value**
WOMAC pain	+ 3.4 ± 14.9	0.99	- 23.7 ± 14.9	0.29	- 20.3 ± 14.9	0.41
WOMAC stiffness	- 0.7 ± 7.1	0.99	- 30.8 ± 17.1	0.21	- 25.8 ± 17.1	0.32
WOMAC function	+ 7.9 ± 14.9	0.91	- 26.0 ± 14.9	0.23	- 21.8 ± 14.9	0.35
WOMAC total	- 4.1 ± 15.3	0.99	- 26.8 ± 16.0	0.26	- 22.6 ± 16.0	0.38
VAS pain	- 10.3 ± 16.4	0.86	- 21.3 ± 16.4	0.44	- 19.7 ± 17.1	0.54
KOOS index	+ 4.8 ± 12.5	0.96	+18.5 ± 12.5	0.34	+20.0 ± 13.1	0.32
SAS index	- 1.5 ± 6.3	0.99	- 7.3 ± 12.2	0.52	- 9.3 ± 6.6	0.38
OARSI/OMERACT responders, %	ND		60.0		60.0	
SF-36						
Physical scale	- 2.1 ± 6.8	0.98	+ 0.6 ± 6.8	0.99	+ 1.9 ± 6.8	0.98
Mental scale	+ 1.4 ± 6.6	0.99	+ 1.3 ± 6.6	0.99	+ 0.5 ± 6.6	0.99

Data are mean ± SD or percentage. ND: not determined.
All indices and scores are on a natural 0–100 mm or normalised 0–100 scale, except the SAS (0-40mm). Baseline values are reported in table 1.
Δ: Mean changes (and standard deviation) from baseline to the different time-point (one week, 3 months and 6 months post-injection) in the OA patients for clinical outcomes parameters.
WOMAC, Western Ontario and McMaster university osteoarthritis; VAS, Visual Analogue Scale; KOOS, Knee injury and Osteoarthritis Outcome Score; SAS, Short Arthritis Assessment Scale; OARSI, Osteoarthritis Research Society International; OMERACT, Outcome measures in rheumatology; SF-36, short-form 36 (quality of life)