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Clinical Study

Randomized clinical trial: expanded autologous bone marrow mesenchymal cells combined with allogeneic bone tissue, compared with autologous iliac crest graft in lumbar fusion surgery

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Abstract

BACKGROUND CONTEXT: Although autogenous iliac crest bone graft (AICBG) is considered the gold-standard graft material for spinal fusion, new bone substitutes are being developed to avoid associated complications and disadvantages. By combining autologous bone marrow mesenchymal stromal cells (MSCs) expanded ex vivo and allogenic cancellous bone graft, we obtain a tissue-engineered product that is osteoconductive and potentially more osteogenic and osteoinductive than AICBG, owing to the higher concentration of MSCs.

PURPOSE: This study aimed to evaluate the feasibility and safety of implanting a tissue-engineered product consisting of expanded bone marrow MSCs loaded onto allograft bone (MSC+allograft) for spinal fusion in degenerative spine disease, as well as to assess its clinical and radiological efficacy.

STUDY DESIGN/SETTING: A prospective, multicenter, open-label, blinded-reader, randomized, parallel, single-dose phase I-II clinical trial.

FDA device/drug status: The investigational experimental product, XCEL-MT-OSTEO-ALPHA, requires the approval of the competent authorities. In this case, approval has been requested from EMA, the European counterpart of the FDA. So far, authorization has not been requested from the FDA because there is no current expectation of placing the product in the United States.

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PATIENT SAMPLE: A total of 73 adult patients from 5 hospitals, with Meyerding grade I-II L4−L5 degenerative spondylolisthesis and/or with L4−L5 degenerative disc disease who underwent spinal fusion through transforaminal lumbar interbody fusion (TLIF).
OUTCOME MEASURES: Spinal fusion was assessed by plain X-ray at 3, 6, and 12 months and by computed tomography (CT) at 6 and 12 months post-treatment. An independent radiologist performed blinded assessments of all images. Clinical outcomes were measured as change from baseline value: visual analog scale for lumbar and sciatic pain at 12 days, 3, 6, and 12 months posttreatment, and Oswestry Disability Index and Short Form-36 at 3, 6, and 12 months posttreatment.
METHODS: Patients who underwent L4−L5 TLIF were randomized for posterior graft type only, and received either MSC+allograft (the tissue-engineered product, group A) or AICBG (standard graft material, group B). Standard graft material was used for anterior fusion in all patients. Feasibility was measured primarily as the percentage of randomized patients who underwent surgery in each treatment group. Safety was assessed by analyzing treatment-emergent adverse events (AEs) for the full experimental phase and appraising their relationship to the experimental treatment. Outcome measures, both radiological and clinical, were compared between the groups.
RESULTS: Seventy-three patients were randomized in this study, 36 from the MSC+allograft group and 37 from the AICBG group, and 65 were surgically treated (31 group A, 34 group B). Demographic and comorbidity data showed no difference between groups. Most patients were diagnosed with grade I or II degenerative spondylolisthesis. MSC+allograft was successfully implanted in 86.1% of randomized group A patients. Most patients suffered treatment-emergent AEs during the study (88.2% in group A and 97.1% in group B), none related to the experimental treatment. X-ray-based rates of posterior spinal fusion were significantly higher for the experimental group at 6 months (p=.012) and 12 months (p=.0003). CT-based posterior fusion rates were significantly higher for MSC+allograft at 6 months (92.3% vs 45.7%; p=.0001) and higher, but not significantly, at 12 months (76.5% vs 65.7%; p=.073). CT-based complete response (defined as the presence of both posterior intertransverse fusion and anterior interbody fusion) was significantly higher at 6 months for MSC+allograft than for AICBG (70.6% vs 40%; p=.0038), and remained so at 12 months (70.6% vs 51.4%; p=.023). Clinical results including patient-reported outcomes improved postsurgery, although there were no differences between groups.
CONCLUSIONS: Compared with the current gold standard, our experimental treatment achieved a higher rate of posterior spinal fusion and radiographic complete response to treatment at 6 and 12 months after surgery. The treatment clearly improved patient quality of life and decreased pain and disability at rates similar to those for the control arm. The safety profile of the tissue-engineered product was also similar to that for the standard material, and no AEs were linked to the product. Procedural AEs did not increase as a result of BM aspiration. The use of expanded bone marrow MSCs combined with cancellous allograft is a feasible and effective technique for spinal fusion, with no product-related AEs found in our study.

Keywords: Bone graft; Clinical trial; Degenerative spondylolisthesis; Expanded stem cells; Lumbar fusion; Mesenchymal stem cells; Mesenchymal stromal cells; Spinal fusion; TLIF; Tissue engineering

Introduction

Low back pain and lower extremity pain due to lumbar degenerative disease and/or spinal stenosis are common problems that many people experience at some point in their life [1]. When conservative treatment fails, spinal fusion surgery is sometimes necessary, but this requires bone tissue grafts or bone substitutes to be implanted between the vertebrae to be fused [2].

Autogenous iliac crest bone grafts (AICBG) are currently considered the gold-standard graft material [3,4] as there is no risk of rejection or transmission of infectious diseases. These grafts have suitable biomechanical, osteogenic, osteoconductive, and osteoinductive properties [5,6]. However, harvesting the graft is associated with acute and chronic complications [7−10] although some authors have concluded that their incidence and severity are overestimated [11]. Still, the supply of autologous bone tissue is limited and its quality depends on individual patient age and biology [7,12]. Furthermore, the failure rate of this technique is not negligible, ranging from 4% to 25% [12].

In an effort to avoid the complications mentioned above, new bone substitutes have been developed and made widely available in recent years [13], but evidence establishing their efficacy is still limited. Synthetic bone grafts generally have poorer fusion rates than AICBG, although clinical outcomes are not significantly different [14]. Bone morphogenetic protein has better fusion results than AICBG and lower reoperation rates, but there are a number of associated complications and treatment costs are high [15−17].

Mesenchymal stem cells have been evaluated for spinal fusion in several animal models with histological and mechanical outcomes comparable to those of iliac crest grafts [18−21]. In the case of humans, published studies

using bone marrow (BM) aspirates with different combinations of stabilizers or carriers have inconsistent results [22]. It is known that MSCs constitute only 0.001 to 0.01% of the total population of nucleated cells in BM aspirate [18], but much higher concentrations can be obtained by culturing MSCs ex vivo [18,23,24]. By combining autologous MSCs expanded ex vivo and allogenic cancellous bone graft, we can obtain a graft material that is just as osteoconductive as AIBCBG, and potentially more osteogenic and osteoinductive owing to the higher concentration of MSCs. This should enable us to avoid donor-site complications, obtain whatever graft volume is necessary in each case, and potentially achieve higher fusion rates than with AICBG.

We present a clinical trial assessing spinal fusion outcomes comparing the use of a tissue-engineered product consisting of ex vivo expanded autologous mesenchymal stem cells seeded onto allogenic bone tissue (MSC+allograft), with the standard surgical procedure using AICBG. The primary objective of our trial was to evaluate the feasibility and safety of MSC+allograft implantation. Our secondary objectives comprised assessment of the efficacy of MSC+allograft according to plain X-rays, computed tomography (CT) scans and clinical criteria: visual analog scale (VAS) for pain, Oswestry Disability Index (ODI) for functionality [25] and Short Form-36 (SF-36) for self-reported quality of life [26,27].

Materials and methods

Study design, randomization, and intervention

We conducted a prospective, multicenter, open-label, blinded-reader, randomized, parallel, single-dose phase I-II clinical trial in which planned enrolment was 62 adult patients with Meyerding grade I-II L4–L5 degenerative spondylolisthesis and/or with L4–L5 degenerative disc disease in need of spinal fusion at 1 or 2 vertebral levels. Patients were enrolled in 5 hospitals from July 2012 to May 2017, after obtaining approval from an independent review board. The trial was registered with EudraCT (2010-02399912) and ClinicalTrials.gov (NCT01552707), and conducted following the principles of the Declaration of Helsinki and the Guidelines of Good Clinical Practice. Inclusion and exclusion criteria are listed in Table 1.

After signing the informed consent form, patients were randomized to one of two study treatment arms by creating a single randomization list for the 62 study patients in blocks of four. Another table was created, also in blocks of four, to replace any randomized patients on whom surgery could not be performed. Competitive recruitment of patients ended once a minimum of 31 patients in each group had undergone lumbar fusion.

Patients in the experimental condition (group A) underwent BM extraction from the posterior iliac crest 21 days before surgery in order to prepare the test product. All patients underwent open TLIF (transforaminal lumbar interbody fusion) after harvesting of AICBG (10 cc for the experimental group and 20 cc for the control group). Pedicle screws were placed, decompression was performed in each patient as needed, the transforaminal approach was used on the most compressed side, and an interbody device was implanted in both groups using 10 cc of AICBG anteriorly.

In the experimental condition, the posterior graft, placed in the intertransverse space on the opposite side to the transforaminal approach, consisted of ex vivo expanded autologous BM-MSCs colonized in allogenic cancellous human bone tissue from a tissue bank. Patients in the control group received AICBG in both the anterior space and the

Table 1
Inclusion/Exclusion criteria

Inclusion criteria:
1) Meyerding grade I-II L4-L5 degenerative spondylolisthesis, alone or involving more than one level, and/or L4-L5 degenerative disc disease, alone or involving more than one level.
2) Age 18 to 85 years (both inclusive).
3) Written informed consent.
4) Ability to understand the nature of the study.

Exclusion criteria:
1) Previous spine surgery.
2) L4 isthmic spondylolisthesis.
3) Smoking more than 10 cigarettes a day.
4) Local or systemic sepsis.
5) Treatment with corticosteroids (oral or systemic) in the 3 months prior to study inclusion or treatment with bisphosphonates for 10 years or more.
6) Positive for HIV-1 or HIV-2, hepatitis B (HBsAg), hepatitis C (anti-HCV-Ab), or syphilis.
7) Pregnancy or intention to become pregnant within the 12 months of signing informed consent, and breastfeeding.
8) Neoplastic disease detected within the last five years or without complete remission.
9) Immunosuppression (unless for diabetes mellitus). The use of immunosuppressive therapy is permitted.
10) Significant abnormal laboratory test results (hematologic and clinical chemistry) contraindicating the surgery.
11) Legally dependent status.
12) Simultaneous participation in another clinical trial or treatment with another investigational medicinal product in the 3 months prior to enrolment in the study.
13) Other conditions or circumstances that might compromise the patient's participation in the study, at the surgeon's discretion.
14) Refusal to take part in follow-up beyond the clinical trial duration.

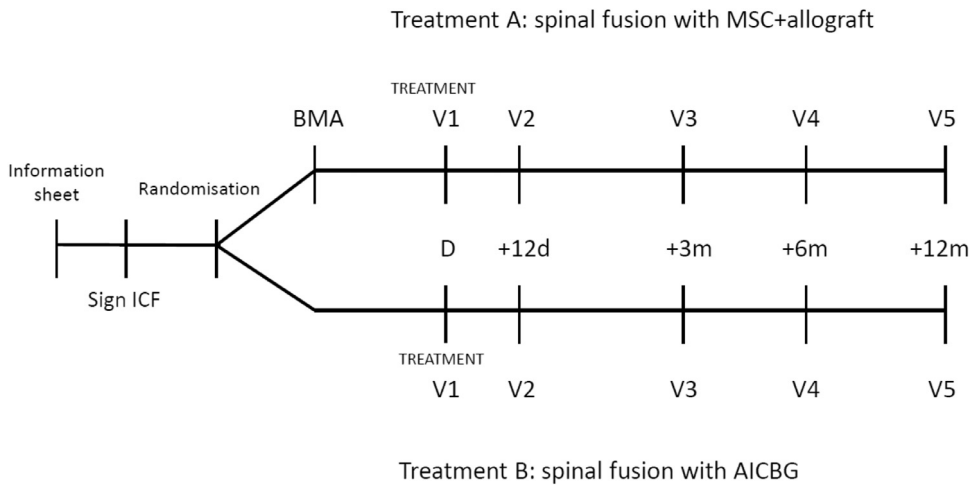

Fig. 1. Study design. ICF, informed consent form; BMA, bone marrow aspiration; V1, Visit 1.

intertransverse space. All patients were followed for 12 months after surgery by their own surgeon at their hospital in the course of scheduled follow-up visits (Fig. 1).

Investigational product

For the BM extraction procedure, patients in group A were placed in a prone position and 120 to 150 mL of BM aspirate were obtained under local anesthesia from one or both posterior iliac crests. The BM aspirate was processed in a clean room at the blood and tissue bank, where multipotent MSCs were isolated from the mononuclear cell fraction of heparinized BM aspirates and subsequently expanded in vitro for 21 days using the methods described by Codinach et al. [28] The final preparation step was colonization into 10 cc of cancellous bone cubes (deantigenized human bone particles) for the last 4 hours [29], before being sent to the patient's hospital for use in surgery.

This tissue-engineered product combines solid particles with a biologically active component consisting of approximately 3×10^5 to 1×10^6 MSCs per cubic centimeter of bone. Its packaging is sterile and nonpyrogenic, suitable for surgical implantation.

The production process was wholly conducted under Good Manufacturing Practice conditions [28]. Characterization of MSCs was based on the criteria established by the International Society for Gene and Cell Therapy [30,31] and an assessment of viability performed following the 7-amino-actinomycin-D exclusion method [31].

Outcome measures

Feasibility was measured as the percentage of randomized patients who underwent surgery in each treatment group and the percentage of patients who had undergone BM aspiration and were ultimately treated with the test product. The protocol also allowed us to ascertain the feasibility of every step in the test workflow, from BM extraction through product manufacturing to graft implantation.

Safety was assessed by analyzing treatment-emergent AEs (TEAEs) for the full experimental phase (defined as all AEs occurring after BM aspiration, in group A, or after surgery, in group B). All AEs and their relatedness to the investigational product according to the treating surgeon's judgment were noted at baseline and at 7 days, 3, 6, and 12 months posttreatment.

An independent radiologist performed blinded assessments of all images. Spinal fusion was assessed by plain X-ray at 3, 6, and 12 months post-treatment using the Molinari fusion scale [32] (Fig. 2). The presence of bridging bone as evidence of spinal fusion, both posterior for the randomized treatment side and anterior, was assessed by CT scan at 6 and 12 months post-treatment (Fig. 3). Complete or partial response to treatment was assessed in this way, with complete response defined as both anterior and posterior fusion, and partial response as either anterior or posterior fusion, but not both.

Clinical outcomes were measured as change from baseline values: VAS for lumbar and sciatic pain at 12 days, 3, 6, and 12 months post-treatment, and ODI and SF-36 at 3, 6, and 12 months posttreatment.

Statistical methods

This trial is a phase I/II study to evaluate the feasibility and safety of treatment as a primary objective. The sample size (n=62) was calculated based on a secondary efficacy variable to detect a statistically significant difference between the two groups for a 1-point change in the Molinari fusion scale compared with baseline for conventional radiography, assuming an alpha of 0.05, a beta of 0.20, and loss to follow-up of 10%. Under the same assumptions, this sample size would allow for a 30-point difference in the percentage of eligible patients who ultimately received the allocated treatment to be statistically significant (eg, 90% vs. 60%).

Feasibility was analyzed for the full randomized population. Safety variables were analyzed for what we termed the

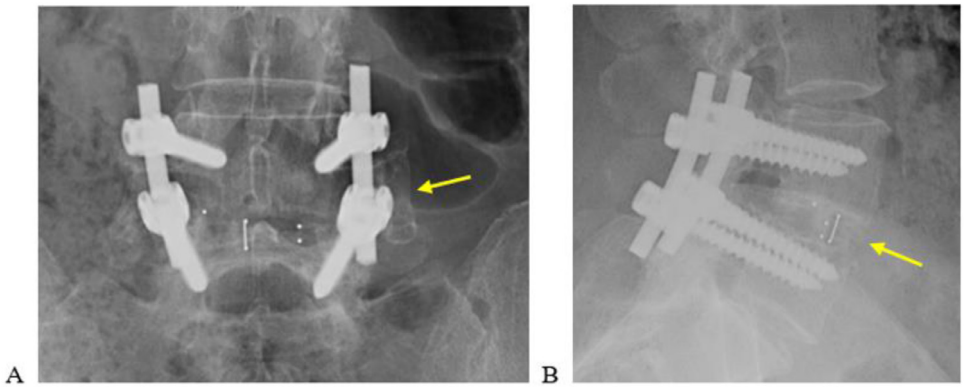

Fig. 2. **X-ray:** (Left) Posterior fusion. (Right) Anterior fusion.

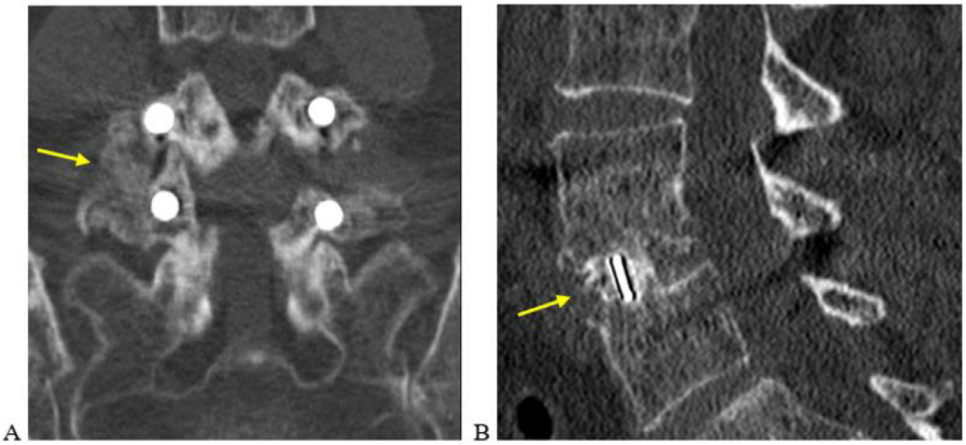

Fig. 3. **CT scan:** (Left) Intertransverse graft on the opposite side to the transforaminal approach. (Right) Interbody graft.

safety population, and efficacy variables for both the safety population and the mFAS (modified Full Analysis Set) population (see Fig. 4 for definitions).

Data by treatment group are presented using descriptive statistics. All hypothesis tests are two-tailed, with significance set at p=.05. For clinical efficacy variables, missing data from VAS, ODI and SF-36 were imputed using the linear interpolation method. For patients lost to follow-up, missing values were imputed using the last observation carried forward method. All efficacy variables were analyzed using prespecified missing data imputation strategies. Identical sensitivity analyzes were also carried out using available data only.

Results

Patients

Demographic characteristics and baseline diagnoses were similar between the two study groups. Most patients were diagnosed with grade I or II degenerative spondylolisthesis. Isolated degenerative disc disease was the diagnosis in 20.6% of patients in group A and 11.4% in group B (Table 2).

Concomitant diseases and comorbidities were present in most patients and were similar for both groups. Recruitment, allocation, exclusion and follow-up are summarized in Fig. 4.

Primary outcome measures: feasibility

The tissue-engineered MSC+allograft product was successfully implanted in most patients in group A (n=31, 86.1% of randomized patients; 91.1% of patients who had undergone BM aspiration). The product had a mean $8.4 \times 10^5 \pm 1.0 \times 10^5$ viable MSCs per cubic centimeter of bone particles (range, $5.8 \times 10^5 - 9.9 \times 10^5$). Three patients did not have enough MSCs for the study graft: BM aspirate cellularity was below the cut-off point in two cases, and MSCs failed to expand in one case. Two patients left the study before BM aspiration: one withdrew consent and the other had to be excluded because of a protocol deviation.

Surgery was also performed as planned in most patients in group B (n=35, 94.6% of randomized patients). Two patients did not receive the surgery: one withdrew consent and the other failed to turn up for surgery.

Primary outcome measures: safety

Most patients who received any treatment experienced TEAEs during the study (88.2% in group A and 97.1% in

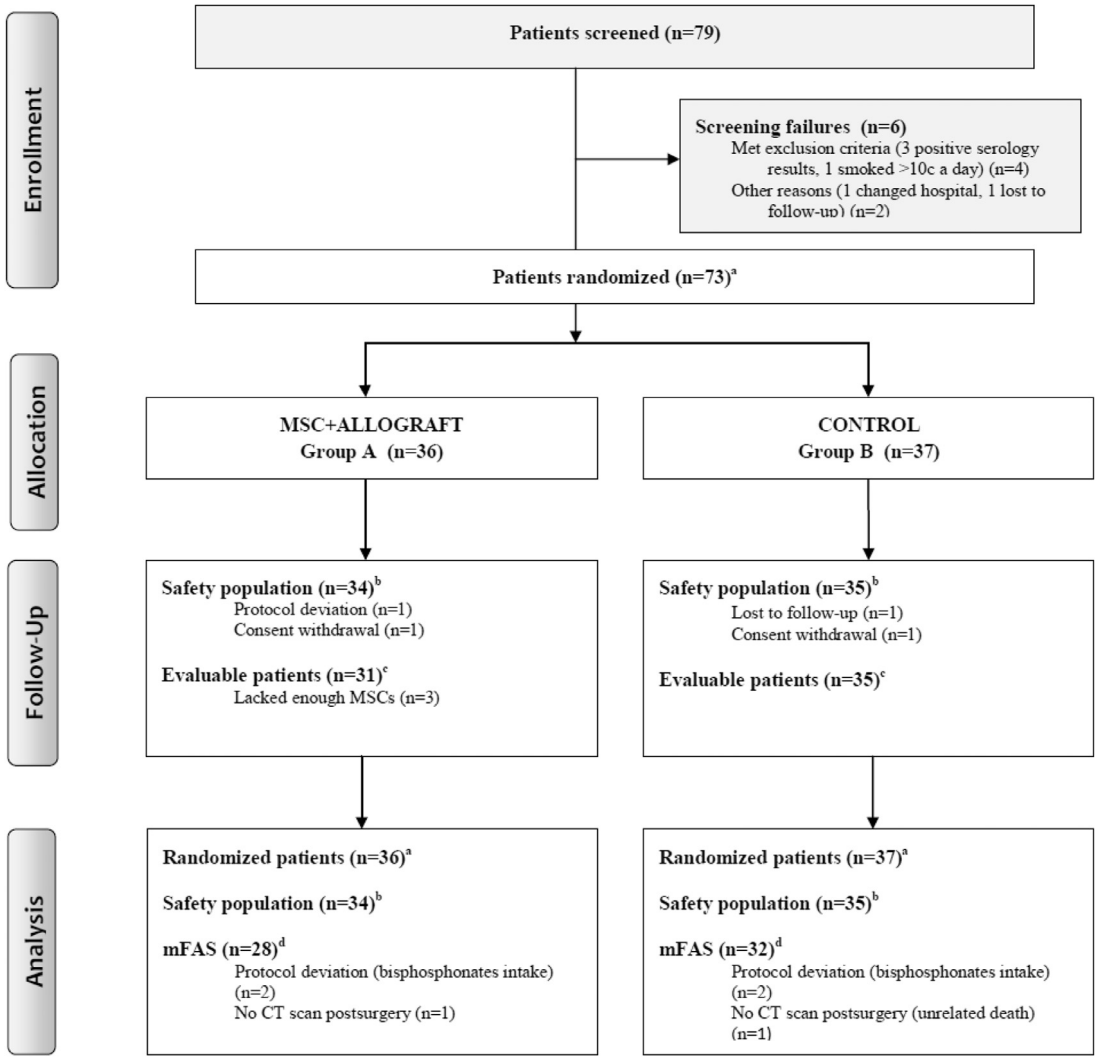

Fig. 4. Study flow chart. [a] Randomized patients: All patients assigned a randomization number. [b] Safety population: Randomized patients who received at least BM aspiration in group A or surgical treatment in group B. [c] Evaluable patients: patients were considered evaluable if they underwent spinal fusion according to the allocated treatment method. [d] Modified Full Analysis Set (mFAS): Evaluable patients that had no major deviation from protocol and had at least one CT-based assessment after treatment.

Table 2
Demographic data and baseline diagnosis

		Group A: MSC+allograft (n=34)	Group B: Control (n=35)	Total safety population (n=69)
Age (y)				
	Mean (95% CI)	61.03 (55.92, 66.13)	60.40 (56.85, 63.95)	60.71 (57.70, 63.72)
	Standard deviation (SD)	14.63	10.32	12.54
	(Min, Max)	(28.00, 85.00)	(32.00, 77.00)	(28.00, 85.00)
	p-value, *t* test			.8375
Gender				
	Male	9 (26.47%)	14 (40.00%)	23 (33.33%)
	Female	25 (73.53%)	21 (60.00%)	46 (66.67%)
	p-value, chi-square			.2333
Baseline diagnosis	L4-L5 degenerative disc disease	7 (20.59%)	4 (11.43%)	11 (15.94%)
	Grade 1 degenerative spondylolisthesis	22 (64.71%)	25 (71.43%)	47 (68.12%)
	Grade 2 degenerative spondylolisthesis	5 (14.71%)	6 (17.14%)	11 (15.94%)
	p-value, chi-square			.5809

p-Value comparison is between treatment arms.

Table 3
Summary of treatment-emergent adverse events (TEAE)

	Group A: MSC+allograft (n=34)	Group B: Control (n=35)
Any TEAE	30 (88.24%)	34 (97.14%)
Number of TEAEs	163	167
Any serious TEAE	5 (14.71%)	3 (8.57%)
Number of serious TEAEs	6	4
Any TEAE that led to death	0 (0.00%)	1 (2.86%)
Number of TEAEs that led to death	0	1
Any TEAE related to the experimental treatment	0 (0.00%)	0 (0.00%)
Any serious TEAE related to the experimental treatment	0 (0.00%)	0 (0.00%)

AE: Any detrimental medical problem, in a patient or subject of a clinical investigation treated with a drug, although it does not necessarily have a causal relationship with said treatment. Any detrimental medical problem, including pain, was reported as an adverse event.

TEAE: any adverse event that started after bone marrow aspiration (group A) or surgical procedure (group B).

Serious TEAE: Any TEAE that is lethal or potentially lethal, makes hospitalization or its prolongation necessary, produces permanent or significant disability, leads to a congenital anomaly or malformation or is a medically relevant event.

One patient in group B died of a lymphoproliferative disorder that was reported in a serious adverse event form.

group B). The most common AEs were back pain (35.3% and 54.3%), procedural pain (35.3% and 31.4%), and arthralgia (20.6% and 22.9%), followed by unspecified pain (17.7% and 8.6%), nausea (14.7% and 8.6%), lower extremity pain (11.8% and 22.9%), paresthesia (11.8% and 8.6%), musculoskeletal pain (11.8% and 5.7%), urinary tract infection (11.8% and 2.9%), and pyrexia (2.9% and 11.4%). BM aspiration did not appear to increase the rate of procedural AEs in group A patients.

Eight patients suffered a serious TEAE: 5 patients (14.7%) in group A (lumbar radiculopathy, postoperative wound infection, ischemic stroke, bile duct stone, and ankle fracture) and 3 patients (8.6%) in group B (lymphoproliferative syndrome resulting in death, postoperative wound infection, and intervertebral disc protrusion in adjacent L3–L4 segment)

None of the TEAEs were considered to be related to the study treatment (Table 3).

Secondary outcome measures: diagnostic imaging

X-ray-based rates of posterior spinal fusion (Molinari grades 1 or 2) were similar at 3 months post-treatment (p=.245), but significantly higher for the experimental group at 6 months (p=.012) and 12 months (p=.0003). In the safety population, most patients in group A showed spinal fusion at 3 months (70.6%), 6 months (67.7%), and 12 months (76.5%) post-treatment. Most of the safety-population patients in the standard treatment group also showed spinal fusion at 3 months (62.9%), but the rate was lower at both 6 months (42.9%) and 12 months (51.4%) (Table 4).

Similarly, CT-scan-based posterior fusion rates were high for group A on the side treated with MSC+allograft at 6 months (82.4%) and 12 months (76.5%) post-treatment. The rates for group B were significantly lower at 6 months (45.7%; p=.0001) and lower, but not significantly so, at 12 months (65.7%; p=.073) (Table 5).

Table 4
X-ray evaluation of posterior spinal fusion

		Group A: MSC+allograft (n=34)	Group B: Control (n=35)	Total safety population (n=69)
		34 (100.00%)	35 (100.00%)	69 (100.00%)
Spinal fusion at 3 months	Yes	24 (70.59%)	22 (62.86%)	46 (66.67%)
	No	4 (11.76%)	8 (22.86%)	12 (17.39%)
	Missing	6 (17.65%)	5 (14.29%)	11 (15.94%)
	p-value, chi-square			.2448
Spinal fusion at 6 months	Yes	23 (67.65%)	15 (42.86%)	38 (55.07%)
	No	3 (8.82%)	11 (31.43%)	14 (20.29%)
	Missing	8 (23.53%)	9 (25.71%)	17 (24.64%)
	p-value, chi-square			.0124
Spinal fusion at 12 months	Yes	26 (76.47%)	18 (51.43%)	44 (63.77%)
	No	0 (0.00%)	12 (34.29%)	12 (17.39%)
	Missing	8 (23.53%)	5 (14.29%)	13 (18.84%)
	p-value, chi-square			.0003

Spinal fusion: Molinari 1 or 2, bilateral or unilateral fusion with trabeculated fusion mass. No spinal fusion: Molinari 3 or 4, lucency or defect in the fusion mass or resorption of graft.

"Missing" label includes both "Not done" and "Missing data."

p-value. Comparison between treatment arms. p-value tests were performed excluding data labelled as "Missing."

Table 5
CT scan evaluation of spinal fusion

		Group A: MSC+allograft (n=34)	Group B: Control (n=35)	Total safety population (n=69)
Posterior spinal fusion at 6 months on side with MSC+allograft or 10 cc of AICBG	Yes	28 (82.35%)	16 (45.71%)	44 (63.77%)
	No	1 (2.94%)	14 (40.00%)	15 (21.74%)
	Missing	5 (14.71%)	5 (14.29%)	10 (14.49%)
	p-value, chi-square			.0001
Anterior spinal fusion at 6 months with 10cc of AICBG	Yes	25 (73.53%)	22 (62.86%)	47 (68.12%)
	No	4 (11.76%)	8 (22.86%)	12 (17.39%)
	Missing	5 (14.71%)	5 (14.29%)	10 (14.49%)
	p-value, chi-square			.2194
Posterior spinal fusion at 12 months on side with MSC+allograft or 10 cc of AICBG	Yes	26 (76.47%)	23 (65.71%)	49 (71.01%)
	No	4 (11.76%)	11 (31.43%)	15 (21.74%)
	Missing	4 (11.76%)	1 (2.86%)	5 (7.25%)
	p-value, chi-square			.0731
Anterior spinal fusion at 12 months with 10cc of AICBG	Yes	27 (79.41%)	26 (74.29%)	53 (76.81%)
	No	3 (8.82%)	8 (22.86%)	11 (15.94%)
	Missing	4 (11.76%)	1 (2.86%)	5 (7.25%)
	p-value, chi-square			.1522

AICBG, autologous iliac crest bone graft.
"Missing" label includes both "Not done" and "Missing data."
p-value. Comparison between treatment arms. p-value tests were performed excluding data labelled as "Missing."

CT-based complete response to treatment at 6 months was significantly higher in group A (70%) than group B (40%) (p=.0038). This significant difference was maintained at 12 months (70.6% in group A and 51.4% in group B; p=.023). The partial or complete response rate at 6 months was also significantly higher in group A compared with group B (85.3% vs. 68.6%; p=.0244), but rates were similar at 12 months (85.3% in group A and 88.6% in group B; p=.616) (Table 6).

Radiological results for the mFAS population were similar to those described for the safety population. (Supplementary Tables S1-S3).

Table 6
CT scan. Percentage of patients with complete or partial response to treatment at 6 and 12 months

		Group A: MSC+allograft (n=34)	Group B: Control (n=35)	Total safety population (n=69)
Complete treatment response at 6 months (both anterior and posterior fusion)	Yes	24 (70.59%)	14 (40.00%)	38 (55.07%)
	No	5 (14.71%)	16 (45.71%)	21 (30.43%)
	Missing	5 (14.71%)	5 (14.29%)	10 (14.49%)
	p-value, chi-square			.0038
Partial or complete treatment response at 6 months (anterior and/or posterior fusion)	Yes	29 (85.29%)	24 (68.57%)	53 (76.81%)
	No	0 (0.00%)	6 (17.14%)	6 (8.70%)
	Missing	5 (14.71%)	5 (14.29%)	10 (14.49%)
	p-value, Fisher			.0237
Complete treatment response at 12 months (both anterior and posterior fusion)	Yes	24 (70.59%)	18 (51.43%)	42 (60.87%)
	No	6 (17.65%)	16 (45.71%)	22 (31.88%)
	Missing	4 (11.76%)	1 (2.86%)	5 (7.25%)
	p-value, chi-square			.0229
Partial or complete treatment response at 12 months (anterior and/or posterior fusion)	Yes	29 (85.29%)	31 (88.57%)	60 (86.96%)
	No	1 (2.94%)	3 (8.57%)	4 (5.80%)
	Missing	4 (11.76%)	1 (2.86%)	5 (7.25%)
	p-value, Fisher			.6159

"Missing" label includes both "Not done" and "Missing data."
p-value. Comparison between treatment arms. p-value tests were performed excluding data labelled as "Missing."

Secondary outcome measures: clinical outcome

The mean baseline VAS score and 95% confidence interval (CI) for lumbar pain and for sciatic pain in the safety population were similar for both groups and indicative of moderate to severe pain (lumbar pain, 6.7 [5.8–7.5] vs. 6.8 [6.0–7.6], p=.859, and sciatic pain, 7.0 [6.1–8.0] vs. 7.3 [6.6–8.1], p=.916, for groups A and B, respectively). Mean lumbar and sciatic pain scores decreased significantly in both cases (p<.0001 for both groups at all time points). No significant differences in change from baseline were observed between the groups at any of these time points.

The mean (95% CI) baseline ODI score in the safety population was also similar for both groups (group A, 40.6 [35.7–45.6] and group B, 43.4 [37.7–49.1], p=.4563), indicating moderate to severe disability. Mean ODI scores decreased significantly in both groups at 3 months (p<.0001 and p=.0003 for groups A and B, respectively), 6 months (p<.0001 and p=.0001), and 12 months (p<.0001 for both groups), but no significant differences were found in the change from baseline at any of these time points.

No statistically significant differences were observed at baseline between treatment groups in any of the eight SF-36 dimensions. The mean (95% CI) baseline PCS (physical component summary) score in the safety population was similar between groups (33.0 [30.7, 35.2] in group A and 29.9 [27.6, 32.2] in group B, p=.0572). A significant increase from baseline, indicating improved physical health, was observed in each treatment group (p<0.0001 for both groups, at all time points). The changes observed in both groups were higher than the minimum clinically important difference for the PCS previously reported for lumbar spine surgery (±4.9 points) [33], and no significant differences were observed in mean PCS change between treatment groups.

No significant differences were found between the groups at baseline for the Mental Component Summary scores (41.6 [37.7–45.5] and 44.2 [39.6–48.9], p=.379). A clear and significant increase relative to baseline, indicative of improvement in mental health, was observed in group A, but not group B, at 3 months (p=.009 and p=.539, respectively), 6 months (p=.001 and p=.546), and 12 months post-treatment (p=.0005 and p=.445). However, there was no significant difference in Mental Component Summary change between groups at any of these time points (p=.193, p=.102, and p=.168 at 3, 6, and 12 months, respectively) (Fig. 5).

Clinical results for the mFAS population were similar to those described for the safety population, using both the linear interpolation method and available data only. (Supplementary Tables S4-S11).

Discussion

Given the drawbacks and limitations of both AICBG and currently available bone graft substitutes for spinal fusion, pursuing an alternative at least as safe and effective as AICBG is of great interest, especially in complex spinal surgery or revision procedures.

MSC+allograft implantation proved to be feasible in the great majority of patients: the product was obtained and implanted in 91% of the patients (31/34) who underwent BM aspiration. Safety was similar to that in our control group, and no TEAEs were found to be related to the investigational product. The overall number of TEAEs was very high for both groups, but most AEs involved pain related to the surgical procedure. Patients are currently under 5-year follow-up for further safety monitoring and no adverse effects associated with the product have been reported to date.

Radiographic posterior spinal fusion rates were similar at 3 months, but significantly higher for the experimental treatment at 6 and 12 months. CT-based posterior spinal fusion rates were also significantly higher at 6 months, though the difference was no longer significant at 12 months. Complete response rates were also significantly higher at 6 and 12 months. This suggests that fusion is achieved earlier and more solidly with the investigational product, a practical advantage in the treatment of complex spine pathology requiring early and solid bone fusion to avoid material fatigue.

The treatment also resulted in improved quality of life and decreased pain and disability for patients, although in this case there were no differences between the experimental and control arm.

Other clinical trials using allograft material with autologous BM aspirate (BMA) or concentrate (BMC) have shown promising results in the past. Taghavi et al. [34] achieved complete solid fusion in all of 7 patients after performing single-level instrumented revision posterolateral lumbar fusion (PLF) using BMA in combination with allograft. Surprisingly, however, only 7 of 11 (63%) of the patients in the same study who received multilevel revision PLF achieved solid fusion. All patients treated with AICBG showed fusion (13 single-level, 11 multilevel). This study reported a much higher fusion rate than ours in both groups, but the patient number was smaller. Hart et al. [35] obtained 15% and 35% fusion rates at 12 months and 24 months, respectively following PLF performed using cancellous allograft chips mixed with BMC. This was compared against cancellous bone chips without BMC, with outcomes clearly supporting the use of BMC, but the fusion rate reported was much lower than ours. Johnson [36] obtained equivalent fusion rates when comparing AICBG side by side with an allograft combined with BMC in intertransverse lumbar fusion.

Likewise, many studies using autograft material with BM have shown encouraging results. Niu [37] published a similar side-by-side study comparing local bone combined with BMA against AICBG, also with similar fusion rates, 90.5% versus 85.7%. Chotivichit et al., [38] however, obtained poorer fusion results in patients with L4-L5 PLF using local bone graft with BMC compared with the same type of graft without BMC. The authors report a few complications linked to BM aspiration, such as local inflammation at the aspiration site, and suggest that the potential for

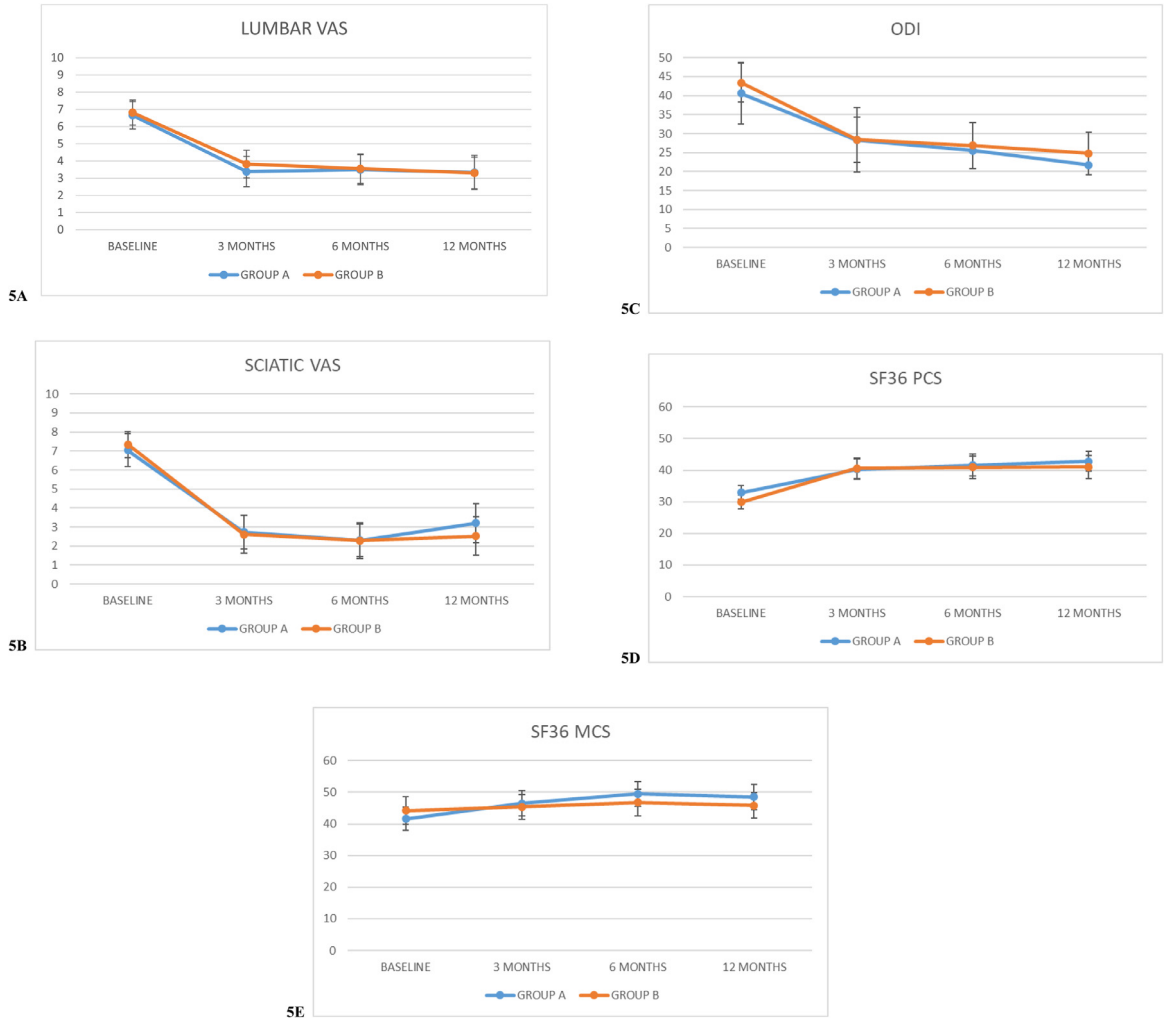

Fig. 5. **Comparison of clinical parameters:** (A) Lumbar Visual Analog Scale (VAS). (B) Sciatic VAS. (C) Oswestry Disability Index (ODI). (D) Short Form-36 Physical component (SF-36 PCS). (E) Short Form-36 Mental component (SF-36 MCS). (95% confidence intervals).

such complications should be considered, even though most are minor. Complication rates related to BM aspiration in large series of hematological patients are very low (.08%) [39], with bleeding in patients with coagulation disorders as the most frequent type. Our own study had no BM aspiration-related complications.

Few clinical trials compare the use of autologous expanded mesenchymal cells with AICBG, and our study is the only one that combines them with allogenic graft material. Recently Blanco et al. [40] published a single-arm, 11-patient clinical trial using expanded autologous MSCs similar to those used in our product, but embedded in tricalcium phosphate, for posterolateral spinal fusion. At 5-year follow-up they had an 80% fusion rate and, as in our trial, found no adverse effects associated with the product; but they had no control group to compare efficacy.

One of the limitations of our study is that we could not assess potential differences in adverse effects or pain attributable to AICBG harvesting, since both groups received AICBG in the intersomatic space. For ethical reasons, the model was designed to ensure fusion using the gold standard for an interbody graft while doing the comparative study in the intertransverse space. If the experimental product had failed to achieve fusion, the patient would still have received AICBG. For the same reason, we did not compare the efficacy of MSC+allograft alone (both posterior and intersomatic) against AICBG alone. Future trials should undertake such a comparison using the study product in the interbody and intertransverse spaces, which could be expected to obtain even better efficacy results and clear differences in complications or postoperative pain, since AICBG-associated complications would be avoided altogether.

One shortcoming of this tissue-engineered product is that it can only be used in elective surgery because of the need for BM aspiration 3 weeks in advance. Allogenic stem cells could circumvent this problem, although adequate evidence of effectiveness and safety is still lacking for such a product [41].

Our goal is to obtain graft material that achieves better and earlier fusion with fewer and less serious adverse

effects than the current gold standard or existing alternatives. This will allow us to treat patients with pseudoarthrosis or those who have undergone multiple previous spine surgery, as well as those with complex spine pathology who require early, stable, solid fusion with a biologically active graft superior to the patient's own bone.

Conclusions

Compared with the current gold standard, our experimental treatment achieved a higher rate of spinal fusion and complete response to treatment at 6 and 12 months after surgery. Furthermore, the treatment clearly improved patient quality of life and decreased pain and disability at rates similar to those for the control arm. The safety profile of the tissue-engineered product was also similar to that for the standard treatment, and no AEs were linked to the product, nor did the BM aspiration step increase procedural AEs. The use of expanded BM MSCs combined with cancellous allograft is a feasible and effective technique for spinal fusion and no product-related AEs were found in our study.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.spinee.2020.07.014.

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