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CLINICAL SCIENCE

Allogenic bone marrow–derived mesenchymal stromal cell–based therapy for patients with chronic low back pain: a prospective, multicentre, randomised placebo controlled trial (RESPINE study)

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ABSTRACT

Objectives To assess the efficacy of a single intradiscal injection of allogeneic bone marrow mesenchymal stromal cells (BM-MSCs) versus a sham placebo in patients with chronic low back pain (LBP).

Methods Participants were randomised in a prospective, double-blind, controlled study to receive either sham injection or intradiscal injection of 20 million allogeneic BM-MSC, between April 2018 and December 2022. The first co-primary endpoint was the rate of responders defined by improvement of the Visual Analogue Scale (VAS) for pain of at least 20% and 20 mm, or improvement of the Oswestry Disability Index (ODI) of 20% between baseline and month 12. The secondary structural co-primary endpoint was assessed by the disc fluid content measured by quantitative MRI T2, between baseline and month 12. Secondary endpoints included pain VAS, ODI, the Short Form (SF)-36 and the minimal clinically important difference in all timepoints (1, 3, 6, 12 and 24 months). We determined the immune response associated with allogeneic cell injection between baseline and 6 months. Serious adverse events (SAEs) were recorded.

Results 114 patients were randomised (n=58, BM-MSC group; n=56, sham placebo group). At 12 months, the primary outcome was not reached (74% in the BM-MSC group vs 69% in the placebo group; p=0.77). The groups did not differ in all secondary outcomes. No SAE related to the intervention occurred.

Conclusions While our study did not conclusively demonstrate the efficacy of allogeneic BM-MSCs for LBP, the procedure was safe. Long-term outcomes of MSC therapy for LBP are still being studied.

Trial registration number EudraCT 2017-002092-25/ClinicalTrials.gov: NCT03737461.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Mesenchymal stromal cells have been shown to reduce disc inflammation and enhance cartilage matrix remodelling.
⇒ Previous clinical trial suggested potential clinical benefit of mesenchymal stromal cell (MSC) intradiscal injection in degenerative disc disease (DDD) but these trials were not conclusive.

WHAT THIS STUDY ADDS

⇒ We conducted a large multicentric randomised placebo-controlled study in DDD using a single intradiscal injection of allogeneic MSCs.
⇒ Our data demonstrate that the procedure is safe. At month 12, we did not demonstrate clinical and imaging benefits as we did not reach our co-primary endpoint.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The RESPINE study provides valuable insights into the complexities of MSC therapy in a challenging clinical context.
⇒ Further research should aim not only to refine MSC therapies but also to explore combinatory approaches that address the multifactorial nature of disc degeneration and chronic pain.

INTRODUCTION

Low back pain (LBP) is the single most common cause for disability in individuals aged 45 years or younger and more than half a billion people are currently suffering worldwide.[1] Chronic LBP limits both quality of life and productivity of patients while increasing the need for access to healthcare.[2] Intervertebral disc degeneration (IDD) is the most significant cause of chronic LBP.[3 4] Current treatment options for LBP due to IDD range from anti-inflammatory drugs to invasive procedures including spine fusion and, more recently, disc

replacement surgery. However, these treatments are symptom-modifying without structural restoration.[5] Recently, there has been a growing interest in developing novel strategies that aim to repair the degenerated disc and restore biological function.[6]

Bone marrow mesenchymal stem/stromal cells (BM-MSC) are skeletal progenitor cells that have the ability to differentiate into various cell types, including bone, cartilage and fat cells.[7] Due to their ability to regenerate damaged tissue and reduce inflammation,[8] BM-MSCs have been investigated as a potential treatment for IDD, a condition characterised by the breakdown of the intervertebral disc (IVD) that cushions the spinal vertebrae.[9 10] MSCs are able to respond to their microenvironment through the secretion of a myriad of biological factors able to modulate immune response, tissue regeneration and repair processes.[11 12] It is commonly acknowledged that these mechanisms may involve the ability of MSC to secrete a large panel of pro-regenerative biological factors directly in the extracellular environment or mediated through the production of extracellular vesicles containing a cargo of growth factors and other molecules capable of stimulating cell proliferation, differentiation and extracellular matrix synthesis. These include members of the transforming growth factor (TGF) superfamily, including TGFbeta and bone morphogenic proteins, hepatocyte growth factor and vascular endothelial growth factor, among others.[13–15] Several in vitro and in vivo studies have been carried out which indicated increased proteoglycan synthesis, reduced levels of proinflammatory cytokines and matrix-degrading enzymes as well as structural benefit (restoration of disc height and imaging scores).[16] However, our understanding of the mechanism(s) of action underpinning the therapeutic effects of MSC in IDD is still incomplete.[6]

Encouraging preliminary results suggested that MSC-based regenerative therapies may provide positive outcomes for this common and debilitating disease. Orozco *et al* conducted a pilot phase I clinical study in patients affected by chronic LBP due to early IDD in a single disc.[17] Patients exhibited rapid and progressive improvement of functional indexes of 65% to 78% over 1 year after intradiscal administration of autologous BM-MSCs. The procedure appeared to be safe and no side effects were reported. The first pilot randomised controlled trial (RCT) to evaluate the efficacy of allogeneic BM-MSC therapy for IDD was conducted by Noriega *et al*.[18] In this phase IIa trial, 24 patients with chronic LBP associated with single level IDD were randomly allocated to BM-MSC intradiscal injection or sham treatment. A significant improvement of pain and functional scores was documented at 3 months and was maintained for at least 1 year in the cell-treated group compared with the control group. In addition, MRI-based IDD scores demonstrated a significant improvement in the treated group. In addition, the Mesoblast trial tested intradiscal administration of allogeneic Stro-1-selected BM-MSCs in a prospective phase II RCT involving 100 patients with chronic, moderate to severe LBP caused by early single level IDD.[19] In this study, patients were randomised to receive one of two different doses of cells and control patients received either saline or hyaluronic acid injection. 69% of the cell-treated groups achieved 50% reduction in pain compared with 31% in the control groups. However, the long-term benefit of the treatment and an assessment of changes in disc water content, a reflection of proteoglycan density, assessed on T2 sequence lumbar MRI, are still lacking.

Here, we report the outcome of a randomised, double-blinded trial in patients with chronic LBP due to single level IDD, persistent for more than 3 months despite conventional medical therapy and without previous surgery. This study evaluated the efficacy of a single intradiscal allogeneic BM-MSC injection versus sham placebo procedure by assessment of pain reduction, functional score and potential change in disc water content after 12 and 24 months.

MATERIALS AND METHODS

Study design

The RESPINE trial was designed as a multicentre randomised, placebo-controlled, double-blind phase IIb trial to compare allogeneic adult BM-MSC therapy and sham-treated controls in subjects with chronic LBP. The clinical trial is registered on EudraCT (number 2017-002092-25) and on clinicaltrials.gov (NCT03737461). All participants provided written informed consent.

Patient selection and enrolment

Participants were recruited by orthopaedic and rheumatology clinicians at six university hospitals in four European countries (France, Spain, Italy and Germany) from April 2018 to April 2021. Patients were selected from a cohort database and by employing print and social media. Eligible participants were aged 18–60 years old, had chronic LBP unresponsive to conservative therapy (including physical therapy and pain medication with level two painkillers[20]) for at least 3 months and had LBP ≥40/100 on a numeric pain rating scale at enrolment. In addition, patients had spine MRI assessment with lumbar IDD grade 4–7 according to the modified Pfirrmann degenerative scale assessed using T2-weighted MRI[21] at one lumbar level from L1 to S1. A second adjacent level of IDD was allowed with a maximum modified Pfirrmann's grade of 4. Use of non-steroidal anti-inflammatory drugs (NSAIDs) was excluded for at least 48 hours and painkillers for 24 hours prior to assessment. The criteria for selection of the disc to receive treatment were defined by the Barcelona centre team considering a sufficient disc space (height loss not below more than 50%), or presence of magnetic remodelling (Modic type I or II changes at the same level of the lumbar disc), and absence of disc herniations (≤3 mm protrusion) with no evidence on imaging of neurological compression. All patients interviewed for eligibility underwent a T2 lumbar MRI in the Radiology Centre at each clinical site. Each T2 mapping MRI was performed using a fast spin echo sequence of the middle sagittal area of the IVD at the time of inclusion and at month 12 and 24 after treatment. The anonymised MRI data were sent to ITRT Barcelona, Spain, by a secure and approved data transfer protocol for analysis. All MRI data were assessed by the same radiologist throughout the trial.

Criteria for exclusion included pregnancy, breastfeeding, congenital or acquired diseases leading to spine deformations (hyperlordosis, scoliosis, isthmus spondylolysis, sacralisation and hemisacralisation), spinal canal stenosis a history of spinal infection, lumbar disc herniation, spinal segmental instability, previous spine surgery or symptomatic posterior lumbo-articular osteoarthritis or predominant facet syndrome on X-ray or MRI (osteophyte and facet hypertrophy), a history of cancer or other malignant condition, an atypical chronic pain syndrome, oral, intramuscular, intravenous or epidural steroid therapy within the previous 3 months prior to treatment injection, a current diagnosis of bleeding disorders and/or taking prescribed anticoagulants that could not be discontinued and an history of allergy to any substances used in the treatments (online supplemental file 1).

Cell production, isolation, expansion and transport

We used allogeneic BM-MSC prepared as described previously.[18 22] Briefly, bone marrow (BM) was aspirated from three healthy volunteers of age 30–50 years, who had consented to the use of their cells for allogeneic patient treatment. BM-MSCs were processed under good manufactoring procedure (GMP) conditions at the Citospin cell production facility (PEI number 15-007) in Valladolid, Spain. Bags containing 100–150 mL of heparinised (BM) were shipped to the facility in a controlled temperature (2–15°C) container, assessed for integrity, weighed and immediately processed in the clean room for isolation and expansion of the cells.

The expansion procedure was performed as previously described.[18 23] Briefly, the mononuclear fraction was isolated by density gradient centrifugation using Ficoll-Paque (GE Healthcare Bio-Sciences, AB, Buckinghamshire, England) and cultured in 175 cm^2 tissue culture flasks (Corning) with cell culture medium consisting of 10% fetal bovine serum, 1% gentamycin in Dulbecco's modified Eagle medium (all from Gibco) and incubated at 37°C under 10% CO_2 until the adherent cells achieved 80% confluence. These cells were characterised by flow cytometry following the most recent update on minimal release criteria for MSC proposed by the International Society for Cell Therapy.[24 25] These criteria refer to positive expression (≥97%) of CD105, CD73, CD90 and CD166 markers and negative expression (≤1%) of CD34 (haematopoietic stem cells and endothelial cells), CD45 (leucocytes and haematopoietic progenitors), CD14 (monocytes and macrophages) and HLA-DR (human leucocyte antigen). These results suggested the presence of MSC and the absence of other cell types in the expanded cell populations. At this point, cells were resuspended in 5% dimethyl sulfoxide or Cryostor CS 5, and were frozen in liquid nitrogen in aliquots of 10 million cells/mL in 2 mL vials until needed. For quality control, these cell stocks were tested on thawing for expression of the same marker panel as well as potency determined in assessing chondrogenic differentiation and cumulative duplications (≤5). Previous data have indicated that cells frozen under these conditions remain stable for at least 5 years.

When a patient was confirmed for inclusion in the cell treatment arm of the study, cells were thawed at room temperature and centrifuged to remove the cryoprotectant. They were then resuspended in fresh culture medium and expanded in culture for 7–10 days as described. Finally, the expanded cell preparations were tested for cell count, viability, mycoplasma, identity, sterility and cumulative duplication. The cell dose was formulated to contain 20 million cells/2 mL of Hypothermosol (Stem Cell Technologies) validated to maintain >85% viability for 72 hours at 2–8°C.[23] The Investigational Medicinal Product Dossier (IMPD) number was elaborated by Citospin and University of Valladolid and was approved by the regulatory authority La Agencia Espanola de Medicamentos y Productos Sanitarios.

Intradiscal injection

On day 0, the treatment administration day, each patient received a 2 mL intradiscal injection of 20 million BM-MSCs in injectable-grade Plasma-Lyte using a 22G spinal needle. This dose was selected based on previous clinical and preclinical studies.[17 26] Under sterile conditions, with the patient in prone position under mild sedation, the intradiscal injection into the symptomatic disc was performed using a right postero-lateral approach under live C-arm fluoroscopy. All injections were performed by the same physician in each hospital to ensure standardisation of technique.

Injection and post-procedure care (anaesthesia and analgesia) were performed in accordance with standard of care as appropriate in the judgement of the treating physician. The injection was performed in the surgical theatre with a recommendation for 24 hours of home rest without specific restriction of activity. All participants were seen 1 week post-injection to check for infection and to evaluate the extent of any post-procedure pain flares. The sham injection without intradiscal puncture consisted on subcutaneous injection in the back of the patient of 2 mL of sterile saline in similar conditions in the surgical theatre.

Randomisation and blinding

Patients were randomly assigned to allogeneic BM-MSC or placebo in a 1:1 allocation using a centralised randomisation system with Ennov software (Clinsight) under the responsibilities of Montpellier University hospital (CHUM). Randomisation was stratified by centre. After BM-MSC therapy, participants attended the clinic and were contacted by telephone to complete the primary safety and efficacy outcome measures at 1, 3, 6, 9, 12 and 24 months post-treatment. The physician in charge of follow-up was different from the surgeon/radiologist who performed the treatment. All participants, assessors, the biostatistician and the physician in charge of follow-up were blinded to the assigned treatment. The surgeon/radiologist who performed the injection was not blinded to the randomisation assignment and did not have any discussion about treatment allocation with patients and clinical observers. Treatment assignment was not revealed until all included subjects had completed 12 months of follow-up. In addition, patients were not unblinded to their treatment assignment if a post treatment intervention was administered.

Outcome measures

The first co-primary endpoint was the efficacy of intradiscal injection of allogeneic BM-MSCs in reducing chronic LBP using the Visual Analogue Scale (VAS) and functional status assessed by the Oswestry Disability Index (ODI)[27] 12 months after treatment, defining strict responders in case of improvement of VAS for pain of at least 20% and 20 mm between baseline and month 12, or improvement of ODI of 20% between baseline and month 12 as shown by the analysis of Ostelo and De Vet.[28] They introduced the concept of a minimally clinically important changes for LBP and suggested that for chronic LBP, a 20 mm change in VAS is a reasonable threshold for significant improvement. This value is based on various studies correlating changes in VAS with global perceived effect scales, establishing that a 20 mm change is both statically and clinically significant.[28]

The secondary co-primary endpoint was the structural efficacy assessed by the disc fluid content measured by quantitative T2 MRI between baseline and month 12. Water content of the discs, determined from T2-weighted sagittal images, was measured in the affected disc segment and in the contiguous 3 to 5 segments above the affected segment. MRI score determination was performed in 5 regions of interest (ROIs) for each disc, 2 for the annulus fibrosus and 3 for the nucleus pulposus. Analysis was performed on the treated disc and two healthy discs as controls. Evaluations were performed before treatment, at 12 and 24 months post-treatment, calculating the T2 relaxation time of each ROI and expressed as a percentage of the initial value.

The secondary endpoints included: (1) VAS, ODI and quality of life (SF-36)[29] at 1, 3, 6, 12 and 24 months; (2) minimal clinically important difference (MCID) on VAS (30% improvement),[28] ODI (10 points improvement),[28] and both compared with baseline; (3) the number of sick leave days among patients with active employment at 12 and 24 months; (4) the consumption of medications to relieve pain (type and dose of painkillers); and (5) the immune response associated with allogeneic cell injection (quantification of anti-HLA antibodies) in all patients. Safety endpoints included the number of adverse events (AEs) and percentage of patients experienced AE, serious AE (SAE) and events of interest related to the procedure such as infection, bleeding, nerve irritation and nerve injury with possible consequences of paresthesia and paralysis.

Assessment of the allogeneic immune response

We evaluated the immunogenicity of the allogeneic BM-MSC treatment by assessing donor specificities of anti-HLA class I and class II antibodies in patients injected with BM-MSCs prior to and 6 months after treatment. DNA was extracted from BM-MSC batches and HLA-A, B, C, DRB1, DRB3/4/5, DQA1, DQB1, DPB1 genotyping was performed using Holotype reagents (Omixon) and the Illumina MiSeq platform. We used the second field resolution level according to the WHO HLA nomenclature (www.HLAnomenclature@hla.alleles.org). At this resolution level, all alleles with the same name code for the same specific HLA molecule. Anti-HLA alloantibodies were then evaluated by single antigen beads using Luminex technology. Briefly, each patient serum was mixed with microbeads coated with a single purified Class l or Class II HLA antigen (Labscreen Single Antigen, One Lambda) and read using a Luminex array analyser (LABScan 200 platform). Reactivity was expressed as raw mean fluorescence intensity (MFI) values and a MFI of 2000 was used as the cut-off for positivity based on historical data and recommendations of Agence de la Biomédecine for organ transplantation to identify the unacceptable donor antigens for a HLA sensitised recipient and to our previous study on BM-MSC-induced alloreactivity.[30]

Sample size calculation

The sample size calculation was based on the findings of the Mesoblast trial.[19] We assumed a clinical responder rate at 12 months of 30% in the control group and 60% in the treatment group. Sample size for the two co-primary endpoints was calculated to obtain a power of 90% leading to a bilateral alpha risk assessment of 5% in balanced groups. At this level, the power of the study was assessed as being in the range 81% to 90%, subject to the co-dependence of the two co-primary endpoints. Taking into account an estimated inclusion failure of 10%, it was necessary to include 56 individuals per group (total 112 subjects).

Statistical analysis

The primary outcome was analysed according to the intention-to-treat principle after a multiple imputation of missing data[31] based on fully conditional specification method. Thirty datasets were imputed corresponding to as many imputations as the percentage of patients with at least one missing data among the variables involved in the multiple imputation (sociodemographic and medical history, current painkillers, pain, disability and quality of life at each visit, randomisation arm). Univariate logistic models were performed to compared primary outcome between groups on each imputed database and summarised using Rubin's rule to obtain the OR, CI and p value of the effect of the intervention on the clinical co-primary outcome.[32]

As we had too many missing data on the anatomical co-primary endpoint, we only performed a descriptive analysis. Sensitivity analysis of the primary outcome and all secondary analyses were performed on the full analysis set, that is, without imputation of missing data, with censoring of data in patients with major protocol deviation, and with analysis in the administration arm.

To analyse the evolution of pain, disability and quality of life throughout the study, we used linear mixed models with random intercept, including discrete time, group and interaction time*group as fixed effects. We computed the adjusted mean differences of scales between each time and baseline, and the p values of these differences were corrected using the false discovery rate algorithm. The same strategy was used with logistic mixed models and adjusted proportion differences for the evolution of the rate of patients achieving the MCID. We used the SAS software V.9.04 (SAS Institute) and R software V.4.3.1.

RESULTS

Demographic and clinical characteristics

An outline of the study design together with the number of participants involved at each timepoint is shown (figure 1). In total, 114 of the 152 screened patients were enrolled between April 2018 and April 2021, and were randomised with 58 patients in the allogeneic BM-MSC group and 56 patients in the sham placebo group. All patients were included in the intention-to-treat analysis and two were excluded in the full analysis set. One patient was excluded because they withdrew consent before injection, and the other because they were unblinded before the end of study. In each group, two patients received the treatment of the other group. We decided to analyse these patients based on the actual treatment they received.

Baseline characteristics of the 114 patients were similar between both groups and are shown in detail in table 1: patients were predominantly male (65%), with a mean age of 40.9 years (± 8.89), mainly currently employed (>90%), had a sick leave due to IDD in less than 25% of cases, had mean pain intensity on VAS of 59.2 (± 16.75) mm and had mean ODI score of 29.9 (± 12.9) on a 0–100% scale.

Primary outcome

At 12 months after the intervention, the percentage of responders was 74% of patients in the experimental group vs 68.8% in the placebo group (table 2). The odds of being a responder for patients in the allogeneic BM-MSC group is 1.23 times higher than for patients in the placebo group (0.32–2.88, p=0.64). At month 12, MRI data were available for 55 patients (30 in the treatment group and 25 in the placebo group). The change in disc fluid content suggestive of disc regeneration between baseline and month 12 was an average of 41.7% in the placebo group vs 37.9% in the treatment group (data not significant, table 3).

Secondary outcomes

For pain assessment using VAS, we observed an improvement in all time points in both groups (table 2). There was no statistically significant beneficial effect of allogeneic BM-MSC on LBP intensity (VAS) at the different secondary time points (1, 3, 6, 12 and 24 months) in the full analysis

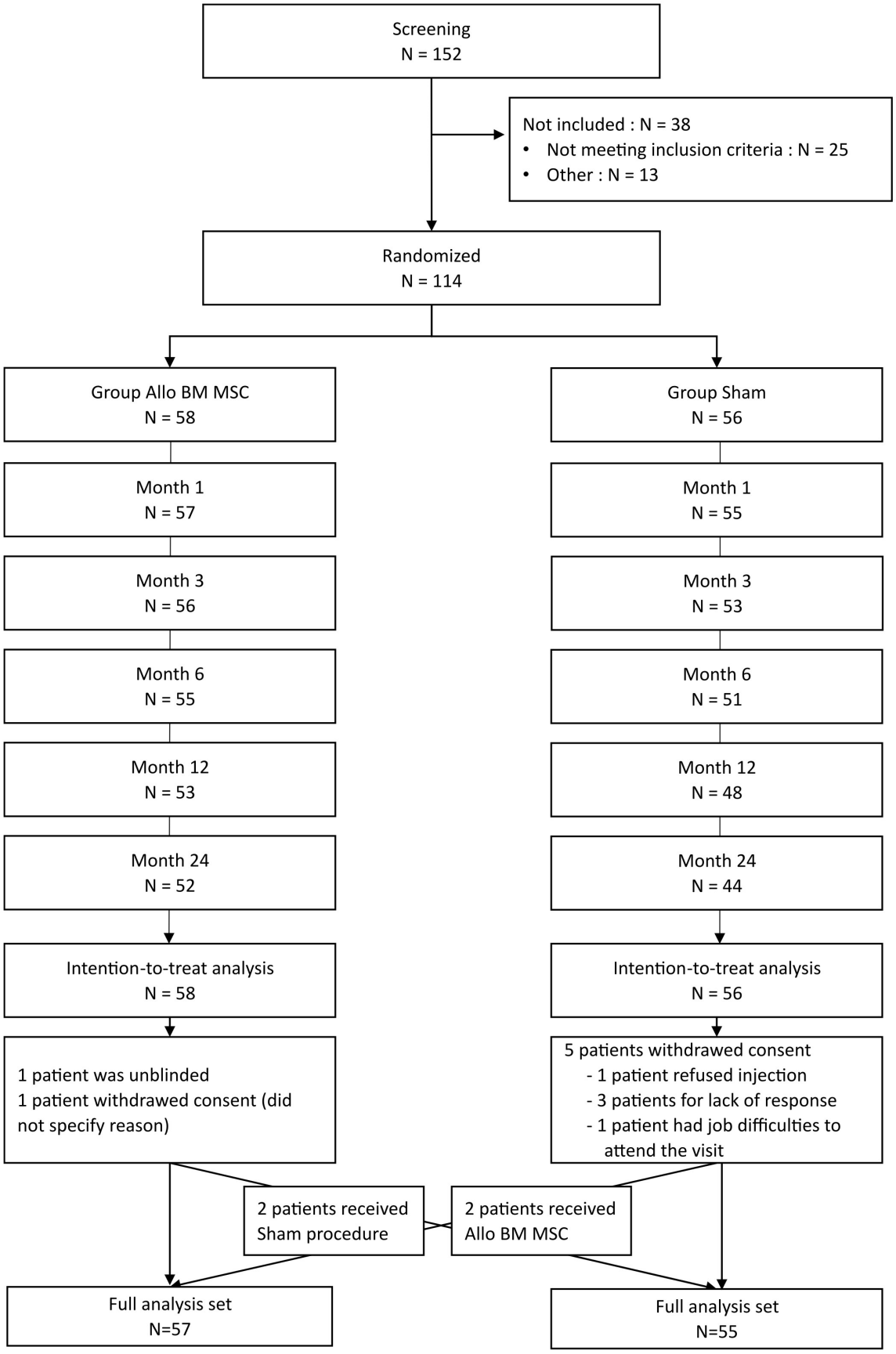

Figure 1 Flow chart of the RESPINE study. BM-MSC, bone marrow mesenchymal stromal cell.

Table 1 Baseline characteristics of patients with chronic LBP associated with IDD

Characteristics	Allogeneic BM-MSC group (N=58)	Sham control group (N=56)	Difference (95% CI)
Mean age (SD), years	42.9 (± 8.8)	38.7 (± 8.6)	4.22 (1.00; 7.43)*
Female, n/N (%)	21/58 (36.2)	18/56 (32.1)	4.06 (–13.33; 21.46)
Active smokers, n/N (%)	15/52 (26.8)	14/50 (25.9)	0.86 (–15.61; 17.33)
Median body mass index (IQR), kg/m²	24.5 (22.9; 26.9)	24.3 (22.6; 27.3)	0.46 (–0.79; 1.75)
Educational level, n/N (%)			
Primary school (at least 5 years of education)	8/56 (14.3)	4/54 (7.4)	6.88 (–4.65; 18.40)
Secondary school (at least 9 years of education)	18/56 (32.1)	18/54 (33.3)	–1.19 (–18.73; 16.35)
College (at least 12 years of education)	11/56 (19.6)	10/54 (18.5)	1.12 (–13.56; 15.81)
License (at least 15 years of education)	10/56 (17.9)	15/54 (27.8)	–9.92 (–25.52; 5.68)
Master (at least 17 years of education)	9/56 (16.07)	6/54 (11.1)	4.96 (–7.80; 17.72)
Doctor (at least 20 years of education)	0/56 (0.0)	1/54 (1.8)	–1.85 (–5.45; 1.74)
Employment status, n/N (%)			
Full-time or part-time employment	54/58 (93.1)	50/55 (90.9)	2.19 (–7.82; 12.21)
Unemployed	2/58 (3.4)	5/55 (9.1)	–5.64 (–14.57; 3.29)
Retired	2/58 (3.4)	0/55 (0.0)	3.45 (–1.25; 8.14)
Sick leave for IDD, n/N (%)	8/33 (24.2)	4/34 (11.8)	12.48 (–5.72; 30.67)
Mean LBP pain intensity on VAS (range, 0–100)	59.4 (± 16.1)	59.1 (± 17.6)	0.33 (–5.92; 6.57)
Median Schober test score (IQR), cm	4.3 (2.5; 5.0)	4.0 (2.5; 5.0)	0.00 (–1.00; 1.00)
Median finger-to-floor test score, (IQR), cm	15.0 (5.0; 25.0)	15.0 (5.0; 25.0)	0.00 (–5.00; 5.00)
Current therapy at baseline, n/N (%)			
Non-pharmacological treatment	9/58 (15.5)	6/56 (10.7)	
Analgesics (all grade) or NSAIDs	24/58 (41.4)	18/56 (32.1)	9.24 (–8.38; 26.85)
NSAIDS or grade1 analgesics	20/58 (34.5)	15/56 (26.8)	7.70 (–9.16; 24.55)
Grade2 analgesics	17/58 (29.3)	11/56 (19.6)	9.67 (–6.00; 25.34)
Mean ODI score (SD) (range 0–100%)	28.9 (± 12.8)	30.9 (± 13.0)	–1.98 (–6.77; 2.81)
Mean ODI score (SD) (range 0–50)	14.5 (± 6.4)	15.4 (± 6.5)	–0.99 (–3.39; 1.41)
ODI subscores, n/N (%)			
No disability (0–4)	2/58 (3.4)	2/56 (3.6)	–0.06 (–6.88; 6.75)
Mild disability (5–14)	28/58 (48.3)	23/56 (41.1)	8.05 (–10.24; 26.34)
Moderate disability (15–24)	23/58 (39.6)	25/56 (44.6)	–4.29 (–22.51; 13.92)
Severe disability (25–24)	4/58 (6.9)	6/56 (10.7)	–3.70 (–14.17; 6.77)
Completely disabled (35–50)	0/58 (0.0)	0/56 (0.0)	NA
Median SF-36 score (SD) (range, 0–100)			
Physical component	37.2 (32.2; 42.0)	36.1 (29.5; 40.0)	–1.30 (–4.07; 1.57)
Mental component	38.2 (33.9; 47.7)	39.5 (30.8; 51.8)	–0.62 (–4.63; 3.93)
Modified Pfirmann degenerative scale, (%)			
Grade IV	10/27 (37.0)	8/27 (29.6)	7.41 (–17.66; 32.48)
Grade V	16/27 (59.3)	1/27 (59.3)	0.00 (–26.21; 26.21)
Grade VI	1/27 (3.7)	3/27 (11.1)	–7.41 (–21.24; 6.42)
Treated vertebral level, (%)			
L3-L4	10.7	3.7	7.01 (–6.48; 20.50)
L4-L5	25.0	29.6	–4.63 (–28.16; 18.91)
L5-S1	64.3	66.7	–2.38 (–27.50; 22.74)

Values are n (%) or mean±SD or median (Q1; Q3).

*Difference statistically significant.
.BM-MSC, bone marrow mesenchymal stromal cells; IDD, intervertebral disc disease; LBP, low back pain; NSAIDs, non-steroidal anti-inflammatory drugs; ODI, Oswestry Disability Index; SF-36, Short Form 36 health survey; VAS, Visual Analogue Scale.

set. At 12 months, the adjusted mean difference in pain VAS was −10.5 (±4.7) mm between the allogeneic BM-MSC group and the placebo group (p=0.15). All the secondary outcomes (ODI, SF-36) showed no significant differences between groups (figure 2, table 2).

The proportion of patients reaching the MCID in VAS pain score (30% improvement) between baseline and 1 3 6 12 and 24 months were slightly elevated in the BM-MSC group but not statistically significant. The same result was seen in the proportion of patients reaching the MCID in ODI score (10 point improvement; table 2). The number of patients on sick leave was similar between baseline, 12 and 24 months (eight patients in the BM-MSC group and four patients in the placebo group). No difference in medication intake, either painkillers or NSAIDs was observed between the two arms throughout the study. Regarding the allogeneic immune response, we found 5 out of 50 patients who developed de novo donor specific antibodies (see in online supplemental tables 6–7). As shown in online supplemental figure 3 and 4, MRI follow-up of the treated disc at 12 months did not find any differences with disc fluid-content and modified Pfirrmann staging between the

Table 2 Evolution of pain, disability and quality of life throughout the study, comparatively to baseline in the full analysis set

Variable	Allogeneic BM-MSC	Sham	Adjusted mean or proportion difference±SD	Corrected P value
Primary outcome				
M1 vs baseline (N=109)	30 (54.55)	21 (38.89)	0.26 (±0.16)	0.46
M3 vs baseline (N=106)	35 (63.64)	32 (62.75)	0.04 (±0.14)	0.77
M6 vs baseline (N=104)	38 (70.37)	32 (64.00)	0.10 (±0.12)	0.77
M12 vs baseline (N=98)	37 (74.00)	33 (68.75)	0.05 (±0.10)	0.77
M24 vs baseline (N=94)	38 (76.00)	33 (75.00)	0.06 (±0.09)	0.77
Secondary outcomes				
Pain VAS at month 1 (N=110)	49.24 (±24.33)	49.80 (±22.65)	−0.55 (±4.49)	0.99
Pain VAS at month 3 (N=106)	45.33 (±25.98)	47.29 (±23.55)	−1.81 (±4.55)	0.99
Pain VAS at month 6 (N=104)	39.72 (±25.87)	41.98 (±24.29)	−2.91 (±4.58)	0.99
Pain VAS at month 12 (N=98)	33.68 (±27.20)	43.06 (±25.12)	−10.55 (±4.68)	0.15
Pain VAS at month 24 (N=94)	31.96 (±25.02)	34.41 (±23.67)	−4.65 (±4.75)	0.99
ODI score at month 1 (N=109)	25.08 (±16.67)	27.81 (±14.95)	−2.24 (±2.88)	0.52
ODI score at month 3 (N=107)	21.45 (±16.07)	23.92 (±15.68)	−2.81 (±2.89)	0.50
ODI score at month 6 (N=105)	18.70 (±13.42)	22.59 (±15.56)	−3.67 (±2.90)	0.42
ODI score at month 12 (N=98)	16.76 (±14.50)	21.08 (±15.63)	−4.39 (±2.95)	0.41
ODI score at month 24 (N=94)	16.23 (±16.07)	19.41 (±15.43)	−4.46 (±2.98)	0.41
MCS (SF-36) at month 1 (N=109)	42.41 (±10.38)	41.45 (±10.92)	1.16 (±2.18)	0.89
MCS (SF-36) at month 3 (N=106)	45.26 (±10.57)	42.15 (±11.05)	3.44 (±2.20)	0.72
MCS (SF-36) at month 6 (N=102)	44.77 (±10.49)	42.72 (±10.58)	2.31 (±2.22)	0.75
MCS (SF-36) at month 12 (N=97)	44.97 (±11.47)	42.67 (±13.04)	2.00 (±2.25)	0.75
MCS (SF-36) at month 24 (N=94)	43.68 (±13.34)	44.06 (±12.22)	−0.67 (±2.27)	0.92
PCS (SF-36) at month 1 (N=109)	38.08 (±7.96)	37.09 (±8.34)	0.75 (±1.69)	0.66
PCS (SF-36) at month 3 (N=106)	40.31 (±8.59)	38.59 (±8.09)	1.52 (±1.70)	0.66
PCS (SF-36) at month 6 (N=102)	41.59 (±9.45)	40.69 (±9.49)	1.05 (±1.72)	0.66
PCS (SF-36) at month 12 (N=97)	43.37 (±10.31)	40.21 (±9.41)	3.27 (±1.74)	0.37
PCS (SF-36) at month 24 (N=94)	42.89 (±9.26)	41.61 (±10.02)	1.82 (±1.75)	0.66
30% Pain VAS improvement, M1 vs baseline (N=110)	20 (36.36)	16 (29.09)	0.09 (±0.11)	0.54
30% Pain VAS improvement, M3 vs baseline (N=106)	21 (38.18)	21 (41.18)	−0.05 (±0.14)	0.71
30% Pain VAS improvement, M6 vs baseline (N=104)	30 (55.56)	22 (44.00)	0.18 (±0.15)	0.35
30% Pain VAS improvement, M12 vs baseline (N=98)	30 (60.00)	18 (37.50)	0.34 (±0.14)	0.08
30% Pain VAS improvement, M24 vs baseline (N=94)	33 (66.00)	26 (59.09)	0.17 (± 0.14	0.35
10-point improvement of ODI, M1 vs baseline (N=109)	13 (23.64)	10 (18.52)	0.05 (±0.07)	0.51
10-point improvement of ODI, M3 vs baseline (N=107)	23 (41.82)	18 (34.62)	0.12 (±0.14)	0.51
10-point improvement of ODI, M6 vs baseline (N=105)	28 (51.85)	22 (43.14)	0.13 (±0.16)	0.51
10-point improvement of ODI, M12 vs baseline (N=98)	30 (60.00)	21 (43.75)	0.26 (±0.15)	0.48
10-point improvement of ODI, M24 vs baseline (N=94)	29 (58.00)	24 (54.55)	0.11 (±0.16)	0.51
30% VAS improvement and 10 points of ODI, M1 vs baseline (N=109)	12 (21.82)	8 (14.81)	0.06 (±0.06)	0.41
30% VAS improvement and 10 points of ODI, M3 vs baseline (N=107)	16 (29.09)	13 (25.00)	0.05 (±0.09)	0.60
30% VAS improvement and 10 points of ODI, M6 vs baseline (N=105)	22 (40.74)	15 (29.41)	0.15 (±0.13)	0.39
30% VAS improvement and 10 points of ODI, M12 vs baseline (N=98)	23 (46.00)	14 (29.17)	0.25 (±0.14)	0.33
30% VAS improvement and 10 points of ODI, M24 vs baseline (N=94)	26 (52.00)	19 (43.18)	0.22 (±0.16)	0.39

Values are n(%) or mean±SD. Adjusted mean (or proportion) differences and their SD are computed in linear (or logistic) mixed models with random intercept, including discrete time, group and interaction time*group as fixed effects. P values are corrected using the false discovery rate algorithm, separately for each outcome.
BM-MSC, bone marrow mesenchymal stromal cell; MCS, Mental Summary Score (SF-36); ODI, Oswestry Disability Index; PCS, Physical Summary Score (SF-36); VAS, Visual Analogue Scale.

2 arms (table 3, online supplemental table 8). In contrast, we observed after 2 years an increase in the water content signal in the BM-MSC group compared with placebo (115% vs 93.2% of initial value, non-significant).

Safety assessment

The treatment groups did not show significant differences in terms of AEs and SAEs. Throughout the study, a total of 488 AEs occurring in 84 patients were reported (272 in the allogeneic BM-MSC group; 216 AEs in the placebo group). The median number of total AE was similar in each group. Causality was assessed as being due to study medication in 20 AEs (17 patients) in the BM-MSC group and 24 AEs (9 patients) in the placebo group. We did not observed AEs such as lumbar surgery, IVD calcification or infectious spondylodiscitis.

A total of 18 SAEs were reported up to 24 months (10 for 7 patients in the BM-MSC group and 8 for 7 patients in the placebo group) with no significant difference between the groups. No SAEs led to discontinuation, and four were considered to be related to the study agent or injection procedure: three patients in the cell-treated group and two in the placebo group experienced serious but transient LBP worsening. The safety profile is summarised in tables 4 and 5.

Table 3 MRI analysis of disc fluid content, in % of baseline disc fluid content and modified Pfirrmann degenerative scale between 12/24 months and baseline

	Evolution of disc-fluid content	Allogeneic BM-MSC	Sham	P value
M12 vs baseline (N=53*)	Mean (±SD)	−5.15 (±20.22)	−1.16 (±18.57)	0.77
	Disc regeneration (evolution>0%)	11 (37.93)	10 (41.67)	
	No evolution (evolution=0%)	1 (3.45)	2 (8.33)	
	Disc degeneration (evolution<0%)	17 (58.62)	12 (50.00)	
M24 vs baseline (N=20)	Mean (±SD)	4.58 (±18.14)	−1.44 (±25.35)	0.55
	Disc regeneration (evolution>0%)	8 (61.54)	3 (42.86)	
	No evolution (evolution=0%)	0 (0.00)	0 (0.00)	
	Disc degeneration (evolution<0%)	5 (38.46)	4 (57.14)	
Changes in modified Pfirmann score between M12 and baseline (N=53*), n/N (%)				0.94
	Improvement	9/29 (31.1)	8/24 (33.4)	
	No change	15/29 (51.7)	11/24 (45.8)	
	Progression	5/29 (17.2)	5/24 (20.8)	

*Two patients were excluded from the analysis because their consent had been withdrawn at 12 months. Values are n (%) or mean±SD.
BM-MSC, bone marrow mesenchymal stromal cell.

DISCUSSION

BM-MSC represents a promising opportunity for the biological treatment of IDD, but only high-quality randomised controlled trials, comparing it to standard care, can determine whether it is a truly effective alternative to spine fusion or disc replacement. The RESPINE trial is one of a few studies investigating the efficacy of allogeneic BM-MSC in the treatment of IDD. The methodology used in the RESPINE double-blind trial was robust. Subjects, radiographic reviewers and rheumatologists assessing the clinical response were blinded to the treatment assignment, thus limiting the source of bias. Only the radiologists in charge of the disc injection were not blinded. We report the first double-blind controlled trial comparing injection of allogeneic BM-MSC to placebo in 114 patients affected by chronic LBP due to single-level IDD. The clinical results demonstrated a progressive improvement of functional and pain indices by 70% within 6 months and by 74–76% at months 12 and 24. The probability of being a responder for patients in the BM-MSC group was higher than for patients in the sham group, although not significant. The MSC-treated group had greater proportions of subjects at most thresholds, but particularly 60% of patients in the BM-MSC-treated arm achieved the pain MCID compared with the control group at month 12. Despite the robust trial design and the favourable premise of MSC therapy, the primary endpoint of significant improvement compared with placebo was not achieved. This outcome necessitates a critical examination of the trial context within the broader landscape of IDD treatments and the implications of our findings. While our study did not conclusively demonstrate the efficacy of allogeneic BM-MSC for IDD treatment, it nonetheless contributes

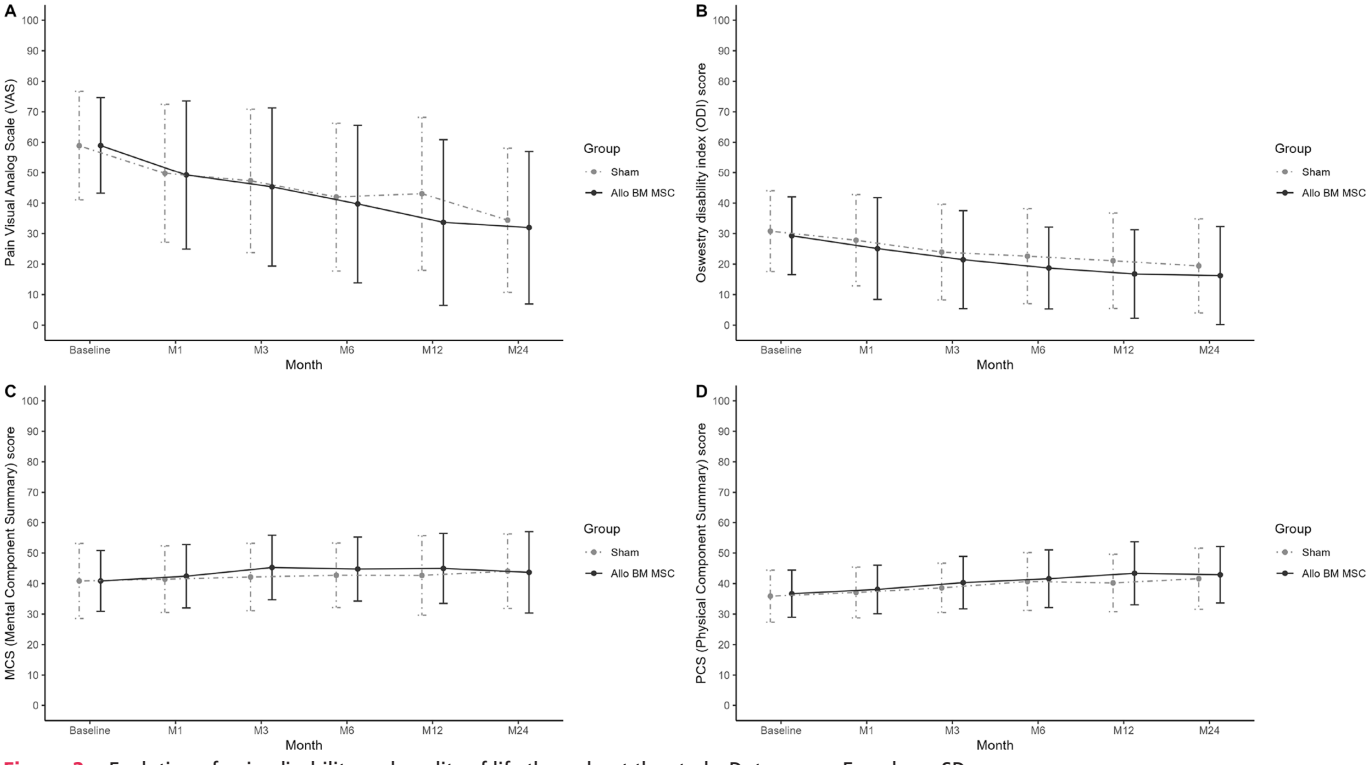

Figure 2 Evolution of pain, disability and quality of life throughout the study. Dots: mean. Error bars: SD.

Table 4 Safety profile during 24-month follow-up

Variables	Allogeneic BM-MSC group (N=58)	Sham control group (N=56)
Time of occurrence after treatment, n/N (%)		
Between screening and baseline	9	10
Before 1 month	49	48
1–3 months	28	29
3–6 months	44	33
6–12 months	53	39
12–24 months	74	40
Type of serious adverse events, n/N (%)		
Hospitalisations for usual care of chronic LBP	0	0
Hospitalisations for events unrelated to chronic LBP	3	5
Events related to chronic LBP without hospitalisation	0	0
Events unrelated to chronic LBP without hospitalisation	0	4
Deaths	0	0
Undefined	5	1

BM-MSC, bone marrow mesenchymal stromal cell; LBP, low back pain.

valuable insights into the complexities of MSC therapy in a challenging clinical context. It is also possible that other cell types, for example nucleus pulposus cells (NPCs) or cell-derived products such as extracellular vesicles (EVs) may provide a more effective outcome. Indeed, Han *et al* demonstrated superiority of NPC over MSC in a rat IDD model.[33] Ambrosio *et al* also demonstrated that NPC-derived EVs were superior to MSC-derived EVs in preserving disc height and preventing degenerative changes in a rat IDD model.[34] These observations point to the criticality of cell type in eliciting a regenerative response in IDD, as discussed by Williams *et al*.[35] However, although rodent studies provide useful information about promising therapeutic strategies, the selection of the optimal cell type can only be determined by patient trials of sufficient scale and robust design. The societal impact of chronic LBP is significant, with incapacity, loss of working days and high expenditure for healthcare. The need for innovative treatments is therefore urgent.

Table 5 Serious adverse events observed during the 24-month follow-up

SAE categories	Allogeneic BM-MSC group (N=8)	Sham control group (N=10)
Musculoskeletal disorders		
Exacerbation of LBP	4	2
Fibromyalgia		1
Hip osteoarthritis	1	
Cervical surgery		1
Fracture		2
Nervous system disorders		
Vagus syndrome		1
Infections		
COVID-19	1	
Oncologic disorders		
Brest cancer	1	
Psychiatric disorders		
Depression		1
Obstetrical disorders		
Pregnancy		1
Caesarean delivery		1
Uterus fibroma surgery	1	

N is the number of serious adverse events. One patient in the allogeneic BM-MSC group and three patients in the sham control group had two serious adverse events.
BM-MSC, bone marrow mesenchymal stromal cell; LBP, low back pain; SAE, serious adverse event.

Our results align with previous clinical studies of intradiscal injection of MSCs for IDD.[17 18 22 36] Orozco *et al* reported that 10 patients suffering from chronic IDD who were injected intradiscally with autologous BM-MSCs exhibited rapid and progressive improvement of functional indexes that approached 65% to 78% by 1 year.[17] Noriega *et al* reported long-term assessments of allogenic MSC injection in single level IDD in 23 subjects.[18 22] In their study, improvements in pain and the ODI persisted 3.5 years later. A large prospective, single-blind, controlled clinical study with allogeneic adult Stro1/3+ mesenchymal precursor cells (MPCs) combined with hyaluronic acid enrolled 100 patients with cLBP caused by moderate single level IDD (modified Pfirrmann score 3–6). Patients were randomised to receive direct intradiscal injections of saline, hyaluronic acid, or two doses of MPCs in a hyaluronic acid carrier, of 6 million or 18 million.[19] Results at month 12 showed that surgical interventions, revealing failure of the treatment, were reduced in the cell-treated groups. In these groups, 62% of the patients achieved a 50% reduction in pain while the control groups achieved only 35%. Functional assessment through the ODI score revealed a greater percentage of patients with at least a 30% reduction in the cell-treated groups (62%) compared with controls (41%). Despite appropriate methodology, the study failed to achieve the primary endpoint. In addition, a recent meta-analysis underlines that MSC-therapy may be effective in relieving pain and improving ODI score significantly in patients with lumbar discogenic pain. They raised that MSC therapy may also be associated with a lower risk of adverse events and reoperation rates.[36]

We observed an increase in the proportion of subjects achieving the MCID composite endpoints for the cell-treated groups compared with the sham at month 12 but did not reach statistical significance, related to the high level of placebo effect. Indeed, the placebo effect has been very strong in studies of other fields where cell therapies were used.[37] The substantial placebo effect observed in our study aligns with existing literature showing strong placebo responses in trials involving pain and mobility assessments. Such effects could overshadow modest but clinically meaningful benefits of new treatments. This phenomenon is particularly notable in IDD, where psychological factors significantly influence pain perception and treatment responsiveness.[38] Future trials should consider methodologies that might better discriminate between placebo effects and the therapeutic action of the treatment, such as more refined patient selection or enhanced blinding and placebo control mechanisms.

Concerning imaging data, lumbar MRI T2 relaxation measurements demonstrated an improvement in the water content of the disc at month 24 but not at month 12, suggesting an increase in proteoglycan and structural improvement in the long term. This is in line with improvements of cartilage signal observed after MSC injection in the knee joint.[39] The failure to meet the primary endpoint brings into question the potency of MSC therapies. While MSC therapies have shown potential in preclinical studies, translating these effects into clinical benefits has proven challenging. This discrepancy could be due to variations in the pathophysiology of IDD among patients, which were not fully accounted for in our trial's design. Future studies might explore

a stratified approach, targeting patient subgroups more likely to respond based on specific biological markers or disease phenotypes. For example, targeting patients with active discopathy (Modic 1 lesions) with low-grade local and systemic inflammation might be more relevant as it is likely to activate BM-MSCs.

Comparatively, our findings contrast with some smaller-scale studies or those using autologous MSCs, which have reported more favourable outcomes. A systematic evidence-based analysis found that cell therapy provided an average reduction of 3.2 points on the pain scale and 27.0 points on the ODI at 1-year follow-up, with a generally good safety profile.[40] Our study achieved a smaller improvement with a reduction in ODI score of 16.8 points at 12 months. This divergence could stem from inherent differences between autologous and allogeneic MSC therapies, including immunogenicity and cell potency issues, which warrant further investigation. In addition, we did not find any differences in clinical results depending on the type of donor. However, it has been established that MSCs are highly heterogeneous between donors, with the consequences of affecting the main functions of MSCs as well as their secretome.[41] The development of cell therapy requires standardisation of procedures to obtain robust clinical results.

The findings reported in this study suggest that there are no apparent safety concerns associated with a single intradiscal injection of MSCs after 24 months of follow-up. Both the procedure and the treatment were well tolerated, with no discitis reported in a total of 58 intradiscal injections. Moreover, there were no clinical symptoms of immune reactions to allogeneic MSCs. There was a low rate of treatment-associated SAEs overall, and the rates of these events in the MSC group were not significantly different from the sham group. The use of allogeneic cells was preferred, as this strategy simplified the overall procedure, improving the yields and decreasing costs.[42] One major risk could be considered the potential immune rejection. However, it has been shown repeatedly that MSCs inhibit immune responses, inducing immunologic tolerance.[43] Allogeneic MSCs have been repeatedly proven in animals over the years without any indication of rejection or delayed immune reactions. In the Poseidon trial, a randomised dose-finding comparison study of allogeneic versus autologous MSCs delivered by transendocardial injection of allogeneic or autologous MSCs, the injection of allogeneic MSCs did not stimulate significant donor-specific alloimmune reactions.[44] In our study, only five patients in the allogeneic group showed sensitisation at the 6-month time point. Our results are in line with the majority of recent clinical trials dealing with allogeneic-MSC showing about 10% of patients with DSA positivity.[30 45]

However, our study had some limitations. We collected only MRI results from 55 subjects across 2 study arms. This resulted in a relatively small number of subjects in each arm, which limited statistical power. The duration of follow-up in our study was another point of concern. While we monitored patients up to 24 months post-treatment, IDD is a progressively degenerative condition, and longer observation periods may be necessary to fully capture the long-term efficacy and safety of MSC therapies. Moreover, a bias in selection of the patients cannot be excluded. Indeed, selection of the patients in the context of cLBP due to single level IDD is very challenging. Several anatomopathological features are recognised as causes of cLBP, some even extrinsic to the spine.[46] For safety reasons, we did not perform discography in this study[47] and therefore we cannot discount the possibility misdiagnosis of patients that received the BM-MSC injection. Therefore, single level IDD may not be the sole cause of LBP in our cohort of patients.

CONCLUSION

In conclusion, while our study did not conclusively demonstrate the efficacy of allogeneic BM-MSCs for IDD treatment, it contributes valuable insights into the complexities of MSC therapy in a challenging clinical context. Our study highlights the overall safety and potential of allogeneic BM-MSC intradiscal transplantation to alleviate LBP. Further research should aim not only to refine MSC therapies but also to explore combinatory approaches that address the multifactorial nature of disc degeneration and chronic pain.

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Clinical Study Protocol RESPINE

A phase 2/3 prospective, multicentre randomized, double-blind trial, comparing intra-discal allogeneic adult BM-MSC therapy and sham-treated controls in subjects with chronic low back pain due to lumbar degenerative disc disease (DDD) unresponsive to conventional therapy

Investigational Product:	**allogeneic BM-MSCs**
Date:	March 16th 2018
Development Phase:	2/3
Brief title:	**Efficacy of intradiscal injection of BM-MSC in subjects with chronic LBP due to lumbar DDD unresponsive to conventional therapy.**
EudraCT number:	2017-002092-25
Sponsor reference code:	UF 9766
Investigators:	Multicenter trial
Sponsor:	CHU Montpellier
Coordinating Investigator/Emergency Contact:	Pr C Jorgensen

This study will be conducted in compliance with the protocol, Good Clinical Pratice and all other applicable regulatory requirements, including the archiving of essential documents.

Table of content

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The signature below constitutes approval of this protocol by the signatory and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol and in accordance with ICH-GCP, the Declaration of Helsinki and local regulatory requirements.

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This protocol was generated in accordance with ICH-GCP.

C. Synopsis

Identifier	**RESPINE:** ***A phase 2/3 prospective, multicentre randomized, double-blind trial, comparing intra-discal allogeneic adult BM-MSC therapy and sham-treated controls in subjects with chronic low back pain due to lumbar degenerative disc disease (DDD) unresponsive to conventional therapy*** **.**
Study design	Multicenter, prospective, double blind, randomized phase 2/3 trial comparing culture-expanded allogeneic adult BM-MSCs with sham-treated controls.
Objectives	***Co-Primary objectives:*** To evaluate the efficay of intradiscal injection of BM-MSCs in reducing chronic LBP using the visual analog scale (VAS) and functional status (by Oswestry Disability Index - ODI) 12 months after treatment, defining responders in case of *improvement of VAS for pain of at least 20% and 20 mm between baseline and month 12, or improvement of ODI of 20% between baseline and month 12*. To evaluate the efficacy of intradiscal inection of BM-MSCs in increasing disc fluid content, measured by quantitative Magnetic Resonance Imaging, between baseline and month 12. ***Secondary objectives:*** ▪ To evaluate the efficacy of intradiscal injection of allogeneic BM-MSCs using VAS and ODI after 3, 6 and 24 months of treatment, defining responders in case of at least 20% of improvement in VAS for pain (with at least 20 mm decrease from baseline on VAS scale) or ODI compared to baseline ▪ Assess efficacy of allogeneic stem cell treatments for DDD on modification of VAS between baseline and 3, 6, 12 and 24 months. ▪ Evaluate modification of disability (ODI),quality of life (SF-36 scores), overall pain intensity, and global assessment by the patient and the physician, between baseline and 3, 6 , 12 and 24 months. ▪ Evaluate modification of affected disc by quantitative Magnetic Resonance Imaging (MRI) signal measurements in T2 and T1spin/echo and T1rho weighted images between baseline, 6, 12, and 24 months used as an indication of disc fluid (T2) and glycosaminoglycan (GAG) content (T1rho) . ▪ Assess modification of employment and work status between baseline and months 12 and 24. ▪ Assess safety and tolerability, measured by the number of participants with adverse events. Adverse events will be reported using clinical review and questionnaires for pain, disability and quality of life along the study. ▪ Assess immune response associated with allogeneic cells injection (quantification of anti-HLA in all patients).

	▪ Assess the medical and non-medical costs
Subjects population	Patients diagnosed with DDD under modified Pfirrmann's score 4 to 6 having persistent LBP that does not respond to conservative treatment (physical and medical) and has lasted at least 3 months. A pain baseline value over 40/100 on visual analogue scale (VAS) will be required. Inclusion criteria will be the following : • Age between 18 and 60 years. • Symptomatic chronic low back pain unresponsive to conservative therapy (including physical therapy) for at least 3 months. • Low back Pain baseline > 40 mm on VAS (0-100). • NSAID washout of at least 2 days before screening • Painkillers washout of at least 24 hours before screening.
Sample size	***112 patients total (12 per participating centre, 16 patients at the coordinating centre)*** -56 patients in the control group (sham treated control) -56 patients in the treatment allogeneic group (allo BM-MSCs) The study is designed to conclude with a Type 1 error of 5%, and a Type 2 error between 10 and 19%. With a power of 90% and 2 balanced groups and according to the literature data (Mesoblast trial 2014), a responder rate is expected at 12 months of the order of 30% in controls and 60% for MSC.. To highlight this difference while justifying a power of 90% and an alpha risk of 5%, taking into account 10% of inclusion failure, it is necessary to include 56 individuals per group a total of 112 subjects. Besides, after Noriega's data, we expect a disc density increase of 22% in cell-treated discs, and 6% in controls, with a standard deviation of 11%. To highlight this difference while justifying a power of 90% and an alpha risk 5% (bilateral hypothesis), taking into account 10% of inclusion failure, it is necessary to include 14 subjects per group. Depending on the dependence of the two co-primary endpoints, the power of the study will be between 81% and 90%.
Statistical methods	The principal analysis will be the intention-to-treat, univariate analysis of the primary outcome, performed after a multiple imputation of missing data. The secondary analysis will include univariate analysis of secondary outcomes at 12 and 24 months, and mixed models to study the modifications of outcomes throughout the trial.
Conduct	**Sponsor:** CHUM **Participation centers (9):** 1. CHUM (FR), 2. CUN (ES), 3. UVA (ES), 4. ITRT (ES), 5. APHP (FR)/2 hospitals, 6. CHU Nantes (FR), 7. BG-BMT

	(DE) 8. UCBM (IT).
Trial duration and timing	First patient in to last patient out: 2.5 years Patients eligible for trial participation according to the inclusion/exclusion criteria will be enrolled over a period of approximately 24 months
Timelines for key study milestones1	• Month 3-6: approval of the Scientific Advisory Board • Month 6-9: VHP procedure for EU approval for allogeneic cells • Month 9: inclusion first patient • Month 33: month 3 of the follow up of the last patient • Month 45: data analysis of all 112 patients *(Month 1= January 2017)*

[1] Key study milestones will be scrutinised during the time course of the project. Significantly delayed key study milestones (e.g. FPFV) might lead to the termination of the grant agreement.

D. Abbreviations

Term	Abbreviation
ADR	Adverse Drug Reaction
AE	Adverse Event
ATMP	Advanced Therapy medicinal product
BM-MSC	Bone Marrow allogeneic mesenchymal cells
CAs	Competent authorities
DDD	Degenerative Disc Disease
DSUR	Development Safety Update Report
ECs	Ethic Commitees
eCRF	electronical Case Report Form
GAG	glycosaminoglycan
GMP	Good Manufacturing Practice
HLT	High Level Term
ICF	Informed Consent Form
IEDSMB	Independent Expert Data and Safety Monitoring Board
IMP	Investigational Medicinal Product
ISF	Investigator Master File
LBP	Low Back Pain
LPLV	Last Patient Last Visit
MAR	Missing At Random
MCAR	Missing Completely At Random
MCH	Major complex histocompatibility
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal stromal stem cell
MSV	Mesenchymal Stem cells of Valladolid
NCA/NCAs	National Competent Authority(ies)
NSAID	Non Steroidal Anti Inflammatory Drug
ODI	Oswestry disability index

Term	Abbreviation
PIL	Patient Information Leaflet
PT	Preferred Term
RR	Relative Risk
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SAP	Statistical Analysis Plan
SESAR	Suspected Expected Serious Adverse Reaction
SOC	System Organ Class
SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
VAS	Visual Analogic Scale

1. Introduction

1.1 Background

1.1.1. Epidemiology and pathogenesis of lumbar DDD

Low back pain (LBP) is the single most common cause for disability in individuals aged 45 years or younger[2]. The costs of back pain in the EU have been estimated to exceed €12 billion each year. Degenerative disc disease (DDD), is the most common cause of chronic LBP[3]. The World Health Organisation (WHO) has included LBP in its list of twelve priority diseases[4]. DDD accounts for 26%-42% of the patients with chronic LBP[3.] DDD is a serious medical and social problem causing chronic back pain, sciatica, spinal stenosis, impaired mobility and lower quality of life. Large surveys show that 14% (13 million) of any patients treated by hospitals had LBP[3,] which accounted for 3% of all emergency visits[5]. Combined physical and medical therapies are temporally successful in relieving pain in approximately 90% of the cases. However, the remaining 10% become chronic and generate a serious public health problem, as chronic low-back pain limits both the quality of life and the productivity of the patient, while increasing the need for health services[6].

1.1.2. Diagnostic of lumbar DDD

Diagnosis of chronic LBP is made with reasonable certainty based on medical history and clinical examination. The patient suffers from persistent LBP more than 3 months, associated with morning stiffness limited in time and reduced functioning. The diagnosis is confirmed by imaging techniques and DDD can be characterized by MRI showing evidence of a correlation between pain and the inflammatory aspect of discopathy [Brinjikji et al., 2015]. Standard therapy of lumbar DDD

Today, no therapy can restore intervertebral disc (IVD) function or provide long-term relief from symptomatic DDD. Conservative therapies (such as rest, behavioural, physical, manual or manipulative therapies, pharmacological agents, and lifestyle modifications) have not proven effective for DDD, but continue to be prescribed during acute episodes of back pain. Likewise, numerous minimally invasive interventional

[2] Fadi Taher, David Essig, Darren R. Lebl, Alexander P. Hughes, Andrew A. Sama, Frank P. Cammisa, and Federico P. Girardi. Adv Orthop. 2012; 2012: 970752. doi: 10.1155/2012/970752. Lumbar Degenerative Disc Disease: Current and Future Concepts of Diagnosis and Management.

[3] Bao-Gan Peng, World J Orthop. 2013 Apr 18; 4(2): 42–52. doi: 10.5312/wjo.v4.i2.42. Pathophysiology, diagnosis, and treatment of discogenic low back pain.

[4] http://www.who.int/medicines/areas/priority_medicines/ch6-13_24/en/

[5] Waterman BR, Belmont PJ Jr, Schoenfeld AJ. Spine J. 2012 Jan;12(1):63-70. doi: 10.1016/j.spinee.2011.09.002. Low back pain in the United States: incidence and risk factors for presentation in the emergency setting.

[6] Palazzo C, Ravaud JF, Papelard A, Ravaud P, Poiraudeau S (2014) The burden of musculoskeletal conditions. PLoS One. Mar 4;9(3):e90633

strategies have failed. These therapies targeted the neural or other biological elements of the disc via, for example, the intradiscal administration of steroids, neurotropic agents or electrothermal energy.

When these treatments fail, several types of surgery are performed to relieve pain and decrease disability. The most common interventions are spinal fusion (arthrodesis), disc surgery (discectomy and sequestrectomy) and ultimately artificial disc replacement. However, DDD surgery is controversial because of its side effects, disturbance of motion and other biomechanical consequences. Notably, surgery can accelerate the degenerative cascade at the pathological disc and at adjacent segments. Moreover, lumbar disc arthrodesis has failed to consistently demonstrate superior outcomes to non-surgical therapies, and has been associated with reoperation rates as high as 26%[7].

1.1.3. Innovative treatment of lumbar DDD

Encouraging results suggest that cell-based, regenerative therapies may circumvent these problems, to provide the world's first effective therapy for this common and debilitating disease. In previous phase 2a clinical trials, patients affected by LBP due to early DDD exhibited rapid and progressive improvement of functional indexes of 65% to 78% over 1 year after intradiscal administration of autologous BM-MSCs (bone marrow mesenchymal stromal/stem cells)[8]. In fact, partner CSP has already trademarked the "MSV" brand for "Mesenchymal Stem Cells from Vallodolid".

In addition, magnetic resonance imaging (MRI) T2 relaxation measurements demonstrated a significant improvement of cartilage signal, in a pilot trial of 12 patients. In a second step, they conducted a single blinded, placebo controlled, phase 2a trial using allogeneic BM-MSCs in 24 patients. The procedure appeared to be safe, and no side effects were reported. The pain score and the Oswestry disability index (ODI) were reduced by about 50% at 6 months following the intervention, while the control, sham-treated patients showed no significant benefit (Noriega et al. Intervertebral Disc Repair by Allogeneic Mesenchymal Bone Marrow Cells: A Randomized Controlled Trial. Transplantation. 2017 Aug;101(8):1945-1951.Transplantation 2017). In a similar US study involving 100 patients with chronic, moderate to severe LBP caused by early DDD, 69% of the cell-treated groups achieved 50% reduction in pain compared to the 31% in the control groups. However, the long term benefit and the structural assessment on MRI was not assessed.

1.1.4. Mesenchymal stromal stem cell (MSCs)

Mesenchymal stem cells, or stromal cells (MSCs) are progenitor cells mainly isolated from adult bone marrow (BM), and adipose tissue. The last two localizations allow easier collection and therefore an extension of their use. They have several functions: synthesis of extracellular matrix, immune tolerance, development, anti-inflammation and fibrosis. MSCs are defined by their functional abilities of differentiation and differ from

[7] Balague F, Mannion AF, Pellise F & Cedraschi C. (2007). Clinical update: low back pain. Lancet 369, 726-728

[8] Orozco L, Soler R, Morera C, Alberca M, Sanchez A & Garcia-Sancho J. (2011). Intervertebral Disc Repair by Autologous Mesenchymal Bone Marrow Cells: A Pilot Study. Transplantation 92, 822-828.

hematopoietic stem cells by the expression of mesenchymal markers (CD105, CD70, CD90), while lacking expression of CD34, CD45, CD14 monocyte or markers of T or B cells, or the major histocompatibility class II (MHC II). MSCs have a phenotypic heterogeneity with some multipotent properties and are the progenitors of multiple lineages including bone, cartilage, muscle or fat. MSCs are currently being studied for tissue engineering applications, including bone and cartilage repair because of their potential to differentiate into different lineages such as chondrocytes, osteoblasts or adipocytes.

MSCs have immunomodulatory and immunosuppressive properties and are involved in both the innate and the adaptive immunity. This immunosuppressive effect is mainly due to the secretion of soluble factors by MSCs and by direct contact with immune cells. MSCs acquire their immunosuppressive properties after exposure to an inflammatory environment. Some cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1-β) or interferon gamma (IFN-γ) are able to activate MSCs.

In tissue repair, MSCs do not seem to have a direct effect, but they stimulate the regenerative properties of resident cells. They have a paracrine effect by reducing the release of pro-inflammatory cytokines and by stimulating the secretion of anti-inflammatory cytokines. MSCs exert their regulatory role by forming a perivascular niche in close contact with endothelial cells and osteoblasts in bone marrow and in close relationship with the immune and hematopoietic stem cells. MSCs operate in collaboration with endothelial factors (epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), stromal cell-derived factor 1 (SDF-1), transforming growth factor (TGF), Angiopoietin 1) to improve their “homing” on damaged sites.

MSC represents a promising opportunity, but only high-quality randomized controlled trials, comparing it to standard of care, can determine whether it is a truly effective alternative to spine fusion or disc replacement. Potential advantages of these treatments are preservation of normal surrounding anatomy, biomechanics, and motion. To accomplish this goal, we propose an ambitious clinical project aimed at developing a broadly available and clinically applicable treatment for DDD. This project will provide that rigorous, clinical proof and will direct future efforts in Europe.

1.1.5. Regenerative therapy based on allogeneic MSCs

MSCs are immune privileged cells due to the low expression of MHC and co-stimulation mechanisms of T cell (CD80/CD86, CD40). Soluble HLA-G also seems to participate in this tolerance. These features help prevent a rapid rejection and immune sensitization. The use of allogeneic cells is preferred, as this choice would widen the horizon of cell therapy and make the treatment available for a much larger population layer by simplifying the procedure, improving the yields and decreasing costs. In allogeneic strategy, one donor will provide the BM-MSC for several recipients, thus the quality controls and the time dedicated to cell expansion are performed once, and reduce of 75% the cost of GMP cell production.

Regenerative stem cell therapies for a number of conditions are in the first phases of clinical trials. There are about 2,000 listed at clinicaltrials.gov (excluding blood stem cell applications). These first phases test only safety; nonetheless, positive signs are often seen. A recent stem cell trial for blindness (macular degeneration) partially restored the

vision of two subjects[9]. Pre-clinical data shows numerous potential advantages of regenerative strategies for treatment, such as preservation of normal surrounding anatomy, biomechanics and motion. Mesenchymal cell therapy has produced exciting results both *in vitro* and *in vivo*. Studies with MSC have been particularly promising[10]. In our previous *in vitro* study, the co-culture of human MSCs with NP cells results in upregulation of both NP cell proliferation and activity and MSC differentiation towards the chondrogenic lineage[11]. Recent research indicates that NP contains populations of MSCs that are very similar to the MSCs recovered from bone marrow[12,13]. Beside chondrogenic potential, MSC have shown an anti-inflammatory effect through the release of IL1RA, TSG6, IDO, iNOS and other anti-inflammatory molecules. MSC are able to inhibit T cell proliferation as well as monocyte activation. This anti-inflammatory effect is important in the benefit observed in DDD where local inflammation is induced by cartilage degradation. Studies in animal models of disc degeneration have shown that MSCs injected in the NP area not only survive for months but also proliferate in canine, porcine and rabbit models[14,15]. In addition, transplanted MSCs induced production of IVD extracellular matrix proteins, including proteoglycan, aggrecan and types I and II collagens[16]. We conducted study were dogs underwent a partial nucleotomy at 3 lumbar levels (L3-L4, L4-L5, and L5-L6); adjacent levels served as nonoperated controls. At 6 weeks, subcutaneous adipose tissue was harvested and MSCs were isolated. The 3 experimental discs that had undergone a partial nucleotomy were randomized to receive: (1) MSCs in hyaluronic acid carrier (Cells/HA); (2) HA only; or (3) No Intervention. Assessments of the 3 experimental discs plus the 2 adjacent untouched discs were made using MRI, radiography, histology, and biochemistry at 12

[9] www.usnews.com

[10] Blanco JF, Graciani IF, Sanchez-Guijo FM, Muntion S, Hernandez-Campo P, Santamaria C, Carrancio S, Barbado MV, Cruz G, Gutierrez-Cosio S, Herrero C, San Miguel JF, Brinon JG & Del Canizo MC. (2010). Isolation and Characterization of Mesenchymal Stromal Cells From Human Degenerated Nucleus Pulposus: Comparison With Bone Marrow Mesenchymal Stromal Cells From the Same Subjects. Spine 35, 2259-2265.

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[12] Peng BG, Pathophysiology, diagnosis, and treatment of discogenic low back pain. (2013) World J Orthop April 18; 4(2): 42-52

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[14] Henriksson HB, Svanvik T, Jonsson M, Hagman M, Horn M, Lindahl A & Brisby H. (2009). Transplantation of human mesenchymal stems cells into intervertebral discs in a xenogeneic porcine model. Spine 34, 141-148.

[15] Yang H, Wu J, Liu J, Ebraheim M, Castillo S, Liu X, Tang T & Ebraheim NA. (2010). Transplanted mesenchymal stem cells with pure fibrinous gelatin-transforming growth factor-beta1 decrease rabbit intervertebral disc degeneration. Spine J 10, 802-810.

[16]Ankrum JA, Ong JF, Karp JM. (2014) Mesenchymal stem cells: immune evasive, not immune privileged Naturebiotech, 32 (3) 252-254

months. Disc repair was clearly demonstrated through histology and biochemical analysis: disc injected with MSC more closely resembled the healthy controls as evidenced in matrix translucency, compartmentalization of the annulus, and in cell density within the NP. Matrix analysis for Type-II collagen and aggrecan demonstrated evidence of a better regenerative stimulation to the disc provided by MSCs when compared to controls[19c]. Finally, these studies also reported that injection of MSCs resulted in better preservation of the height and water content of discs. Further proofs of NP regeneration have been obtained in another large size animal study (sheep) conducted by members of the consortium (UCBM). A nucleotomy model has been used to test MSCs transplantation with cell dose escalation. We have delivered 100µl of the hyaluronan based hydrogel with a suspension of low or high doses of BM-MSCs, using the nucleotomy model without injection as control. We have shown that a high dose of autologous MSCs ($1x10^7$ cell/ml) regenerates the NP in this model with high cell engraftments up to one year after transplantation [unpublished data].

More than 450 trials are ongoing in 2016 using allogeneic MSCs in regenerative medicine worldwide, with major focus on cardiovascular pathology, chronic inflammatory diseases and musculoskeletal pathologies, based on allogeneic cells in most cases[17]. We propose a large-scale double blind controlled trial to validate DDD therapy based on BM-MSC.

1.2 Clinical trial rationale

The Advanced Therapy (ATMP) medicinal product described in the present study is MSV, acronym for "Mesenchymal Stem cells of Valladolid", produced at the Institute of Molecular Biology and Genetics (IBGM) and processed under Good Manufacturing Practices (GMPs).

In its early description (Refs 18 and 19) (OROZCO L, SOLER R, MORERA C, ALBERCA M, SÁNCHEZ A, GARCÍA-SANCHO J. Intervertebral Disc Repair by Autologous Mesenchymal Bone Marrow Cells: A Pilot Study.) 92: 822-828; MSV term was used for cells obtained from the patient himself for autologous use. Afterwards, MSV cells from a healthy donor have been used for allogeneic use (Eudra-CT: 2011-005321-51) [19a, 19b]

In our pioneering open pilot study in humans[18], we treated 10 patients suffering chronic DDD with autologous bone marrow MSC. Feasibility and safety were confirmed and strong indications of clinical efficacy were identified. Patients exhibited rapid and progressive improvement of functional indexes that approached 65% to 78% by 1 year (***Figure 1***)[19]. In addition, MRI T2 relaxation measurements demonstrated a significant

[17] Imran Ullah*, Raghavendra Baregundi Subbarao* and Gyu Jin Rho. Human mesenchymal stem cells - current trends and future prospective. Bioscience Reports (2015) 35, 1-18

[18] Orozco et al, 2011http://www.citospin.com/MESODISC/2011_Orozco_Transplantation_MSV&LBP_short.pdf

[19] Orozco L, Munar A, Soler R, Alberca M, Soler F, Huguet M, Sentis J, Sanchez A & Garcia-Sancho J. (2013). Treatment of Knee Osteoarthritis With Autologous Mesenchymal Stem Cells: A Pilot Study. Transplantation 95, 1535-1541.

improvement of cartilage signal, in 11 of 12 patients (Figure 3), suggesting increase in proteoglycan and water content.

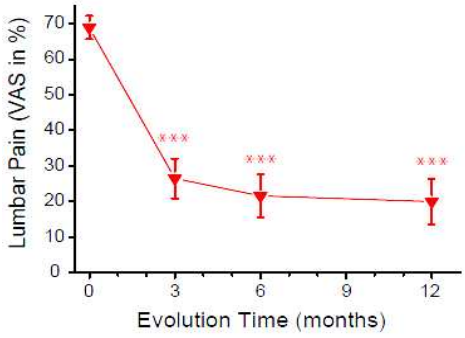

Figure 1. Lumbar pain assessment over time after MSC injection in the disc[18]

In parallel to clinical improvement, the MRI T2 relaxation imaging measurements demonstrated a significant improvement of cartilage signal, in 11 of 12 patients, suggesting increase in proteoglycan and water content.

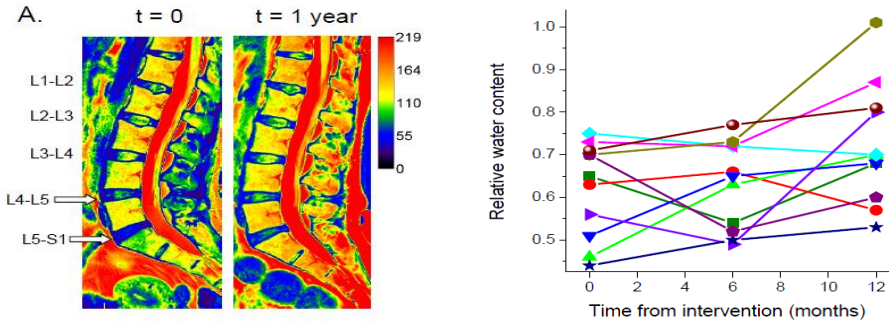

Figure 2. MSC treatment increases water content of the treated disc. A: improvement of L4-L5 and L5-S1 discs. B: increase in water content in 12 patients at one year[18].

Mesoblast reported the completion of a prospective, multicenter, double blind, controlled clinical study with allogeneic adult Mesenchymal Precursor Cells (MPCs) combined with hyaluronic acid (HA) in United States and Australia. The MPC proposed by Mesoblast

[19a]Vega A, Martin-Ferrero MA, Del Canto F, Alberca M, Garcia V, Munar A, Orozco L, Soler R, Fuertes JJ, Huguet M, Sanchez A & Garcia-Sancho J. (2015). Treatment of Knee Osteoarthritis With Allogeneic Bone Marrow Mesenchymal Stem Cells: A Randomized Controlled Trial. Transplantation 99, 1681-1690.

[19b]Noriega DC, Ardura F, Hernandez-Ramajo R, Martin-Ferrero MA, Sanchez-Lite I, Toribio B, Alberca M, Garcia V, Moraleda JM, Sanchez A & Garcia-Sancho J. (2016). Intervertebral disc repair by allogeneic mesenchymal bone marrow cells: a randomized controlled trial. Transplantation. in press DOI: 10.1097/TP.001484

[19c] Meisel HJ, Ganey T, Hutton WC, Moseley T, Hedrick M, Strem B (2009) Intervertebral disc repair using adipose tissue-derived stem and regenerative cells: Experiments in a canine model
Eur Spine J 18, Suppl. 4, 467

are precurcors derived from BM of health donors sorted by Stro1/3 antigen. These cells have not been compared to allogeneic cryopreserved MSC, and are protected by the Australian company. The biological potential of cryo-preserved MPC is decreased compared to our BM-MSC expanded for only 2 passages, and non-frozen cells.

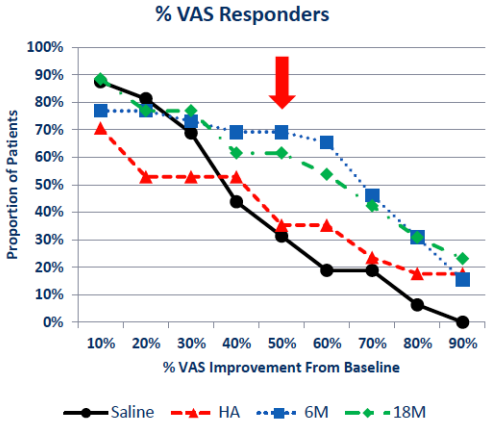

Figure 3. VAS improvements were observed in Mesoblast clinical study[20]

The trial enrolled 100 patients with chronic moderate to severe low back pain caused by early disc degeneration (<30% disc height loss, 83% below Pfirrmann Grade 5 by MRI). Patients were enrolled across 13 sites and randomized to receive direct intra-discal injection of saline, hyaluronic acid or two doses of MPCs in hyaluronic acid carrier, 6 million or 18 million. Results at month 12 showed that surgical interventions (revealing failure of the treatment) occurred in 25.0% in Saline treatment, 10.0% HA, 6.9% 6M and 3.3% in the 18M groups. The differences among cell-treated and control groups were statistically significant, in the cell-treated groups 62% of the patients achieved 50% reduction in pain while the control groups achieved only 35% (p = 0.036) (Figure 5). Functional assessment through the ODI score revealed a greater percentage of patients with at least ≥30% reduction in the cell-treated groups (62%) compared to controls (41%). However, the long term benefit and the structural assessment on MRI was not described.

Another randomized and controlled trial[21] was performed by one of the partners of the consortium. This study included 24 patients with chronic back pain diagnosed with lumbar disc degeneration with intact annulus fibrosus. The patients were randomized into two groups, one control and the other treated with 20 million cells per disc. A 12-month follow up has already been completed for 22 patients without any serious adverse effect report. The pain score was reduced by near 50% (p<0.01) at 6 months from the intervention while the control sham-treated patients showed no significant effects. The ODI was reduced similarly.

Based on these previous data**, RESPINE aims to bring this innovative therapy to the clinic in order to rapidly (within 3 months) and sustainably (for at least 24 months)**

[20] NIH, Safety and Preliminary Efficacy Study of Mesenchymal Precursor Cells (MPCs) in Subjects with Chronic Discogenic Lumbar Back Pain, ClinicalTrails.Gov, 2011, http://www.clinicaltrials.gov/ct2/show/NCT01290367?term=back+pain+stem+ cell&rank=1.

[21] https://clinicaltrials.gov/ct2/show/NCT01860417?term=garcia-sancho&rank=1

improve DDD patients' quality of life while relieving their pain and disability. Specifically, we aim to assess the efficacy of a new MSC-based therapy for discogenic LBP, persistent despite conventional medical therapy. We will validate this advanced cellular therapy through a randomized, double blind controlled, phase 2b clinical trial in order to define the efficacy of allogeneic MSC therapy vs placebo and sham injection in a 2 year study to demonstrate long term clinical and structural benefit. The efficacy of the regenerative therapy will be assessed via a rigourous, controlled double blind phase 2b clinical trial, in terms of visual analogue scale (VAS), ODI questionnaire, SF-36 scores and MRI at month 24. This study is essential for future broadening of cell therapy applications.

1.3 Justification of the route administration, dosage and treatment period

Allogenic BM-MSCs will be administered via imaging control into the disk affected by DDD where they are expected to exert their therapeutic effects. As we mentioned above, no current standard treatments are available to influence the course of the disease and no drugs/devices brought significant proofs to limit the structural cartilage degradation in DDD. In the previous studies, we injected a median dose of 25.10^6 of cells with a good outcome and without side effects. Thus, we decided to use a dose of $20.\ 10^6$ in the active arm and a sham procedure in the control group without active product.

Based on previous recommended papers, it seems the best option to check efficacy in chronic LBP is a period of more than 6 months. Thus, we retained as primary outcome an improvement of pain and functional status 12 months after treatment.

1.4 Risks/Systemic effects

The number of trials have risen rapidly investigating the efficacy in treating conditions such as type I and II diabetes, liver cirrhosis and regeneration, fistulas, cardiovascular disease (ClinicalTrials.gov Identifier NCT00999115), limb ischemia, amyotrophic lateral sclerosis and lipodystrophy (http://clinicaltrials.gov). More than 400 trials have been conducted with more than 1000 patients treated with MSC. Furthermore, BM-MSCs are also under examination in clinical case studies for graft-versus-host disease immunosuppression, rheumatoid arthritis, Crohn's disease and ulcerous colitis, multiple sclerosis, soft tissue augmentation and bone tissue repair (Fang et al., 2006, 2007; Leblanc et al., 2008) with a clinical benefit, as well as appropriated tolerance. Clinical experience is available with both autologous and allogeneic BM-MSCs administration.

Systemic effects: The most obvious disadvantage of using allogenic cells is the possibility of host immune rejection. MSC, however, are immune privileged[Erreur ! Signet non défini.] or immune evasive[16] and inhibit immune responses in a manner not restricted by the HLA system. As a result, non-matched MSC are better tolerated than other cell types. In fact, there are no reports of rejection in animal experiments[22,23,24] and studies

[22] Yan H & Yu C. (2007). Repair of full-thickness cartilage defects with cells of different origin in a rabbit model. Arthroscopy 23, 178-187.

of transplanted MSC persistence show the same values for autologous and allogeneic cells[16,25]. In humans, excellent tolerance to allogeneic MSCs has been reported in many clinical trials. For example, in a recent meta-analysis of 87 lupus erythematosus patients, no transplantation-related adverse events were found after 4-years of follow up[26]. Similarly, no transplantation-related adverse events occurred in MSC-treated patients with breast cancer[27], left ventricular dysfunction[28], ankylosing spondylitis[29], graft versus host disease[30], and other autoimmune diseases[31]. Finally, safety of allogeneic MSC has also been demonstrated in humans after systemic or local

[23] Shimomura K, Ando W, Tateishi K, Nansai R, Fujie H, Hart DA, Kohda H, Kita K, Kanamoto T, Mae T, Nakata K, Shino K, Yoshikawa H & Nakamura N. (2010). The influence of skeletal maturity on allogenic synovial mesenchymal stem cell-based repair of cartilage in a large animal model. Biomaterials 31, 8004-8011.

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[25] Muschler GF, Nakamoto C & Griffith LG. (2004). Engineering principles of clinical cell-based tissue engineering. J Bone Joint Surg Am 86-A, 1541-1558.

[26] Wang D, Zhang H, Liang J, Li X, Feng X, Wang H, Hua B, Liu B, Lu L, Gilkeson GS, Silver RM, Chen W, Shi S & Sun L. (2013). Allogeneic mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus: 4 years of experience. Cell Transplant 22, 2267-2277.

[27] Koc ON, Gerson SL, Cooper BW, Dyhouse SM, Haynesworth SE, Caplan AI & Lazarus HM. (2000). Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy. J Clin Oncol 18, 307-316.

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[29] Wang P, Li Y, Huang L, Yang J, Yang R, Deng W, Liang B, Dai L, Meng Q, Gao L, Chen X, Shen J, Tang Y, Zhang X, Hou J, Ye J, Chen K, Cai Z, Wu Y & Shen H. (2013). Effects and Safety of Allogenic Mesenchymal Stem Cells Intravenous Infusion in Active Ankylosing Spondylitis Patients Who Failed NSAIDs: A 20 Week Clinical Trial. Cell Transplant.

[30] Lazarus HM, Koc ON, Devine SM, Curtin P, Maziarz RT, Holland HK, Shpall EJ, McCarthy P, Atkinson K, Cooper BW, Gerson SL, Laughlin MJ, Loberiza FR, Jr., Moseley AB & Bacigalupo A. (2005). Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. Biol Blood Marrow Transplant 11, 389-398.

[31] Bernardo ME, Pagliara D & Locatelli F. (2012). Mesenchymal stromal cell therapy: a revolution in Regenerative Medicine? Bone Marrow Transplant 47, 164-171.

administration for treatment of cardiovascular diseases without reported adverse effects after four-year follow-up[32].

Local effects: the complications are the same as in the discography, and rarely presented. The most common complaint is the exacerbation of pain for 1-2 weeks, which is usually solved by analgesia and muscle relaxants for a short period. The most serious complication is discitis, occurring in less than 0.1 % (Osti et al., 1990). Procedure related adverse events such as of infections, bleedings, nerve irritations and nerve injuries with its possible consequences paresthesia and paralysis are exceptional during the injection using the CT scan fto guide the radiologist, but patients will be informed.

1.5 Benefits

The objective of this clinical trial is to generate efficacy and tolerability profiles of single injections of one dose of allogenic BM-MSCs versus standard of care (placebo), when administered locally into the lumbar disk affected by DDD after *in vitro* cell expansion. The potential of BM-MSCs to lead to a disease-modifying therapeutic option for the treatment of this chronic and debilitating disease will be assessed by MRI after 6 month, 1 and 2 years.

1.6 Risk-benefit assessment

This study will progress beyond the current state of the art as it will be the first European clinical trial to reach significance in assessing allogeneic BM-MSCs for the treatment of DDD. This study provides the first major step in determining subsequent clinical and commercial activity relating to stem cell therapy. It will definitively provide robust in patient regenerative medicine research that either supports or refutes the potential of intradiscal injection of BM-MSCs for the treatment of LBP due to intervertebral disc degeneration grade 3-6 modified Pfirrmann grading syste. If successful, this will allow a new therapy to be taken to the next level of testing, taking the field closer to marketability and delivery, a key step that has eluded stem cell therapies to date.

Based on previous clinical experience with similar products reported in the literature, BM-MSCs is deemed to be effective and safe in various diseases. Manufacturing of the study medication is performed according to GMP standards. The risk of local or systemic infection, bacteremia, or sepsis due to contamination of the cell preparation seems negligible. Pre-clinical *in vitro* and *in vivo* evaluations of the study medication did not show any hint of tumorigenic potential of the preparation or systemic migration of BM-MSCs after intra-discal injection. There is a low risk of systemic AEs occurring after the end of the observation period, but the sample size of this clinical trial implies a very low probability of detecting rare events anyway. At last, procedure related adverse events such as of infections, bleedings, nerve irritations and nerve injuries with its possible consequences paresthesia and paralysis are very rare as the injection are performed using the CT scan to guide the radiologist, but patients will be informed.

The benefit for patients with chronic LBP may be considerable, since BM-MSCs might represent the first disease-modifying therapeutic option for this chronic and debilitating disease. This clinical trial will be accompanied by a Data and Safety Monitoring Board

[32] Peeters Peeters CM, Leijs MJ, Reijman M, van Osch GJ, Bos PK. (2013) Safety of intra-articular cell-therapy with culture-expanded stem cells in humans: a systematic literature review. Osteoarthritis Cartilage 21: 1465-1473

(IEDSMB), which will review safety data and provide recommendations to the Sponsor regarding the safety of subjects, the conduct of the study and potential premature termination. Furthermore, under certain pre-defined conditions, e.g. the occurrence of suspected unexpected serious adverse reactions (SUSARs), the Sponsor will suspend treatment of subjects until a decision whether to continue the clinical trial or not has been taken in accordance with the recommendations of the IEDSMB. In conclusion, subjects will be exposed to limited risks during their participation in this clinical trial.

Therefore, the risk-benefit ratio for this clinical trial is anticipated to be favourable and advocates its conduct in the selected group of subjects. Members of the consortium carried out a pilot trial demonstrating the feasibility and safety of the procedure[33,34]. The currently proposed trial would be performed by these experienced researchers who have already developed cell therapy materials and protocols with the following characteristics. On the basis of the previous authorizations from the Spanish Medicines Agency for GMP fabrication of bone marrow autologous MSC, bone marrow allogeneic MSC[35] and use for IVD in two pilot clinical trials with autologous[36] and with allogeneic cells[37,38], RESPINE project will follow a common protocol, substantially identical to that already approved.

Risk minimisation plan:

the sham treatment in the control arm is not harmful to the patient : no disc injection, no placebo injection, no local anaesthesia will be performed in the control patients. The patients with DDD are chronic patients, painful but with no vital risk or no significant risk of delaying effective therapy. The 6 months follow up with conventional therapy is not a reduction of chance to DDD cure s it is a slow irreversible disc degeneration. Conventional treatment as physiotherapy or analgesic is allowed during the trial period. Moreover, in case of severe pain, the patient will be allowed to perform surgery, he will be considered as a failure. We enclose adequate risk minimization measures in the protocol avoiding the inclusion of patients who are not eligible to the intravertebral disc surgery or so painful that they have high probability to undergo surgery in the next 6 months period. Conventional treatment as physiotherapy or analgesic is allowed during the trial period. In case patients became painful and who develop “peripheral neurological deficit” they will undergo electromyography of the legs. In case the results obtained show pinched sciatic nerve, the

[33] Orozco L, Soler R, Morera C, Alberca M, Sanchez A & Garcia-Sancho J. (2011). Intervertebral Disc Repair by Autologous Mesenchymal Bone Marrow Cells: A Pilot Study. Transplantation 92, 822-828.

[34] Orozco L, Munar A, Soler R, Alberca M, Soler F, Huguet M, Sentis J, Sanchez A & Garcia-Sancho J. (2013). Treatment of Knee Osteoarthritis With Autologous Mesenchymal Stem Cells: A Pilot Study. Transplantation 95, 1535-1541.

[35] http://www.citospin.com/MESODISC/Certif_GMP.pdf

[36] http://www.citospin.com/MESODISC/AutorizacionesECs/AEMPS_Disc_allo_MSV_2012.pdf

[37] Vega A, Martin-Ferrero MA, Del Canto F, Alberca M, Garcia V, Munar A, Orozco L, Soler R, Fuertes JJ, Huguet M, Sanchez A & Garcia-Sancho J. (2015). Treatment of Knee Osteoarthritis With Allogeneic Bone Marrow Mesenchymal Stem Cells: A Randomized Controlled Trial. Transplantation 99, 1681-1690.

[38] Noriega DC, Ardura F, Hernandez-Ramajo R, Martin-Ferrero MA, Sanchez-Lite I, Toribio B, Alberca M, Garcia V, Moraleda JM, Sanchez A & Garcia-Sancho J. (2016). Intervertebral disc repair by allogeneic mesenchymal bone marrow cells: a randomized controlled trial. Transplantation. in press DOI: 10.1097/TP.001484

investigator will decide to exclude the patients from clinical trial and shift the patient to the appropriate standard of care (AINS, steroid injection, rest, physiotherapy). In case of severe neuropathic pain (sciatica), the patient will be allowed to perform surgery, he will be considered as a failure. We enclose adequate risk minimization measures in the protocol avoiding the inclusion of patients who are not eligible to the intravertebral disc surgery or so painful that they have high probability to undergo surgery in the next 6 months period as well as patients with herniated discs.

2. Study design and endpoints

2.1 Study design

RESPINE is a phase 2/3 efficacy multicenter, prospective, **randomized, controlled double blinded trial**, comparing intra-discal allogeneic adult BM-MSC therapy and sham-treated controls in subjects with chronic low back pain (>3 months) due to lumbar degenerative disc disease (DDD) unresponsive to conventional therapy. Patients will be randomized in 2 arms of 56 patients and followed up for 24 months.

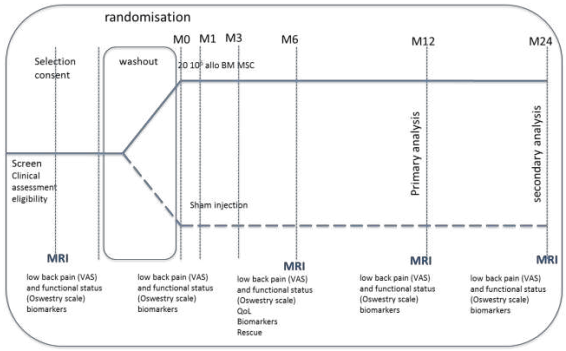

Figure 4- Study design

RESPINE will use bone marrow allogeneic mesenchymal cells (BM-MSCs) from donors volunteers expanded according to validated and approved IMPD, using same cell process as in a previous assay authorized by EMA and performed by members of the consortium. Bone marrow will be obtained from healthy donors after informed consent signature

2.2 Randomization

The randomization will be centralized and stratified on the investigation center, and on the current attending to a rehabilitation programm at the inclusion visit. It is performed 24 hour-a-day online by the central randomization web-service customized by the **IZKS Mainz**. To randomize a patient, investigators have to login, enter the patient identification, additional checks and factor values. Since patients are assigned to the centre, the randomizing investigator is currently in. The patient identification must be unique within all randomizations performed by the randomizing investigator's centre. If everything is entered correctly, a summary of the patient data including the randomization result is shown. For double-blind trial the randomization result will be a treatment code. The Investigator/surgeon in charge of injections will not have any discussion about treatment allocation with patients and clinical observers. The PI won't have access to the list of treatment codes used for blinding. Thereby, **IZKS Mainz** will transmit the treatment code of the patient to each local pharmacy.

2.3 Primary and secondary objectives

- Co-Primary objectives: the study will be considered a success only if all the separate endpoints are statistically significant (Multiple Endpoints in Clinical Trials, Guidance for Industry, FDA 2017). Indeed, we aim to demonstrate an effect on two critically important features : the clinical feature, and the radiological feature.

To evaluate the efficacy of intradiscal injection of allogeneic BM-MSCs in reducing chronic low back pain due to lumbar DDD using the visual analog scale (VAS) and functional status assessed by the Oswestry disability index (ODI) after 12 months of treatment, defining a responder as a patient with an improvement of VAS for pain of at least 20% and 20 mm between baseline and month 12, or with an improvement of ODI of 20% between baseline and month 12.

To evaluate the efficacy of intradiscal injection of BM-MSCs in increasing disc fluid content, measured by quantitative Magnetic Resonance Imaging, between baseline and month 12.

- Secondary objectives:

- To evaluate the efficacy of intradiscal injection of allogeneic BM-MSCs using VAS and ODI after 3, 6 and 24 months of treatment, defining responders in case of at least 20% of improvement in VAS for pain (with at least 20 mm decrease from baseline on VAS scale) or ODI compared to baseline
- Assess efficacy of allogeneic stem cell treatments for DDD on modification of VAS, considered as a continuous measure, between baseline and 3, 6, 12 and 24 months.
- Evaluate modification of disability (ODI) and quality of life (SF-36 scores), considered as continuous measures, between baseline and 3, 6 , 12 and 24 months.
- Evaluate modification of affected disc by quantitative Magnetic Resonance Imaging (MRI) signal measurements in T2 and T1spin/echo and T1rho weighted images between baseline, 6, 12, and 24 months used as an indication of disc fluid (T2) and glycosaminoglycan (GAG) content (T1rho) .
- Assess modification of employment and work status between baseline and months 12 and 24.
- Assess safety and tolerability, measured by the number of participants with adverse events. Both the acute and long-term safety will be analysed. The incidence of potentially procedure related adverse events such as infections, bleedings, nerve irritations and nerve injuries with its possible consequences paresthesia and paralysis will be evaluated. Adverse events will be reported using clinical review and questionnaires for pain, disability and quality of life along the study.
- Consumption of medications to relieve pain such as type and dose of analgesics will be evaluated. Paracetamol (acetaminophen) and levels 2 analgesics will be assessed throughout the study at each visit.
- Assess immune response associated with allogeneic cells injection (quantification of anti-HLA in all patients).

3. Selection of Subjects/population(s) and patient follow up

This study will include patients diagnosed DDD in the lumbar spine and persistent low back pain that do not respond to conservative treatment (physical and medical) lasting at least 3 months. A pain baseline value over 40/100 on visual analogue scale (VAS) shall be required. Criteria will be most astringent in order to guarantee homogeneity among the participating centres.

3.1 Inclusion criteria

- Age between 18 and 60 years.
- Symptomatic chronic low back pain unresponsive to conservative therapy (including physical therapy performed during at least 1 month before inclusion and pain medication with level 2 analgesics in failure or intolerant to level during at least 1 month) for at least 3 months.
- DDD assessed by (Pfirrmann's score modified Griffith et al) grade 4 to 7 at one level. If second level, it should be adjacent (Pfirrmann's score 1-4 maximum)
- Low back Pain baseline > 40 mm on VAS (0-100).
- NSAID washout of at least 2 days before screening
- Painkillers washout of at least 24 hours before screening

3.2 Exclusion criteria

- Congenital or acquired diseases leading to spine deformations that may upset cell application (hyperlordosis, scoliosis, isthmus lesion, sacralization and hemisacralization).
- Symptomatic posterior lumbo-articular osteoarthritis or predominant facet syndrome on Xray or MRI (osteophyte and facet hypertrophy).
- Prior to the screening visit, has received:
  - Oral corticosteroid therapy within the previous 3 months, OR
  - Intramuscular, intravenous or epidural corticosteroid therapy within the previous 3 months
- Spinal segmental instability (defined by lumbar dynamic X-Ray in extension/flexion with antero-post translation > 3 mm and/or angular mobility > 15°).
- Spinal canal stenosis (Schizas score > B).
- History of spinal infection.
- Lumbar disc herniation with non truncated sciatica or cruralgia, as well as lumbar cysts and radiculopathy
- Previous discal puncture or previous spine surgery.
- DDD on 3 levels, or DDD on 2 levels but not adjacent, or DDD with modic 2 or 3 phases
- Patients not eligible to the intravertebral disc surgery
- Patients who have the risk to undergo a surgery in the next 6 months

- Obesity with body mass index (BMI in Kg/size in m^2) greater than 35 (obesity grade II).
- Participation in another clinical trial or treatment with another investigational product within 30 days prior to inclusion in the study.
- Abnormal blood tests: hepatic (alanine aminotransferase [ALT] and/or aspartate aminotransferase [AST] >1.5 × upper limit of normal [ULN]), renal, pancreatic or biliary disease, blood coagulation disorders, anemia or platelet count of <100 × 10^9/L.
- Significant medical problems, such as uncontrolled hypertension, symptomatic heart failure; or any other clinically relevant condition or current medication that in the opinion of the investigator contra-indicates the use of any of the study or rescue medications.
- Pregnant or lactating women, or premenopausal women not using an acceptable form of birth control, are ineligible for inclusion. Specific recommendations for contraception and pregnancy testing is included in the information provided in the IB.Contraception will be maintained during treatment and until the end of relevant systemic exposure. Additional pregnancy testing will be performed at the end of relevant systemic exposure.The patients will be required to use contraception for 2 years until the study has completed.
- In each case of delayed menstrual period (over one month between menstruations) confirmation of absence of pregnancy is strongly recommended. Methods that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods. Such methods include:

combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation : oral, intravaginal, transdermal

progestogen-only hormonal contraception associated with inhibition of ovulation : oral, injectable, implantable

intrauterine device (IUD)

intrauterine hormone-releasing system (IUS)

bilateral tubal occlusion

vasectomised partner

sexual abstinence are ineligible for inclusion.

- Positive serology for following infection: Syphilis, HIV, Hepatitis B, or C.
- Contraindication to MRI assessed by the investigator.
- Intolerance or allergy to local anaesthesia.
- Any history of Cancer or immunodeficiency disease.
- Previous transfusion or transplantation.
- Current sick leave due to work accident .
- Prisoners or subjects who are involuntary incarcerated
- Patients subject to legal protection measures

3.3 Assignment of intervention and blinding

Treatment allocation will be performed 24 hour-a-day online by a central randomization web-service (**IZKS Mainz**). To randomize a patient, investigators have to login, enter the patient identification, and check the inclusion and exclusion criteria. The patient identification must be unique within all randomizations performed by the randomizing investigator's centre. If everything is entered correctly, a summary of the patient data including the randomization result is shown. For double-blind trial the randomization result will be a treatment code. The PI won't have access to the list of treatment codes used for blinding. Thereby, the treatment code of the patient will be transmited to each local pharmacy.

Blinding or masking will be carried out at all stages of packaging and conditioning for shipping following the treatment allocation. Injections used for all groups will be clear and indistinguishable from each other..

During the application of the cells, in the control group, patients will receive the sterile vehicle using similar procedure by unblinded radiologist (sham procedure without discal injection). The investigator in charge if patient inclusion and follow-up (different from the physician injecting the cells) and the patient will be blinded

3.4 Patient follow up/Monitoring

Patient follow up will be performed at 1, 3, 6, 12, 24 months including Clinical parameters (VAS, ODI, SF-36) and MRI at months 6, 12 and 24. Immunomonitoring will be performed at inclusion or baseline, months 1, month 6

3.5 Withdrawal of the clinical trial

Patients may always and without specification of reasons withdraw their informed consent and, as a corollary, withdraw from the trial related therapy or the entire clinical trial. The investigator also has the right to withdraw patients in agreement with the sponsor or his delegate. The IEDSMB also has the right to advise the sponsor and investigator to withdraw patients from the trial in the event of concurrent illness, adverse events, and treatment failure after a prescribed procedure, protocol violations, or other reasons as ccurrence of a pregnancy, an exclusion criteria, which would interfere with the patients safety according to the investigator.

Patients must be withdrawn from the study under the following circumstances:

- The patient withdraws consent,
- Loss of contact to the patient after 3 reminders,
- Development of an intercurrent illness or condition, which would interfere with the patients continued participation,
- Occurrence of a SAE or an exclusion criteria, which would interfere with the patients continued participation,

Discovery that the subject entered the clinical trial in violation of the protocol or occurrence of a significant protocol violation during the clinical trial. The withdrawal will be decided after notice of the principal investigator and methodologist.

Other reason in the opinion of the investigator that cells should not be injected to prevent the patient from harm.

Any situation that in the opinion of the investigator would pose inacceptable risks to the patient if trial participation is continued.

For any discontinuation the investigator should obtain all required details and should document the date and reason of the premature termination in the CRF.

If the reason for withdrawal is an AE, the specific event will be recorded in the eCRF. The investigator will make thorough efforts to document the outcome.

If a patient is withdrawn from the clinical trial before injection of the cells, it will be replaced by another eligible patient.

If a pregnancy occurs, the patient will be withdrawn from the clinical trial and will be closely followed up.

3.6 Lost to follow up

Investigators should make every effort to minimize the number of patients lost to follow-up and to obtain a maximum of information on patients lost to follow-up, particularly in the search for any AEs. Contact details from investigator centre will be given to each patient. The investigator will call the patient before each scheduled visit. If necessary, the investigator could contact General Practitioner (GP) to reach the patient.

3.7 Follow up after discontinuation

If a patient discontinues from the study for an AE, this patient must be followed up until LPLV and until resolution or stabilization of the event.

As far as possible, in case of withdrawal, the investigator will perform all examinations scheduled for the final study visit, which includes recording of AEs. In any case, the patient will be treated in accordance with standard care in the centre.

3.8 Replacement of patients

Drop-outs will not be replaced (the sample size has been increased assuming a drop-out rate of 10%).

If a patient is withdrawn from the clinical trial before injection of the cells, it will be replaced by another eligible patient.

3.9 Follow up after termination

Patients will be followed up for 5 years long term after study termination for safety assessement.

4. Measurement of outcomes

4.1 Primary Outcome Measures:

The clinical response is defined as pain relief of at least 20% and 20 mm decrease on VAS scale between baseline and month 12, or 20% improvement of functional index ODI at month 12 compared to baseline. Chronic low back pain is assessed using the VAS pain scale (0 – 100 , where 0 represents no pain and 100 represents the worst pain imaginable). ODI scale ranges from 0 to 50 and allows evaluation of disability (0% – 20%: minimal disability; 20% – 40%: moderate disability; 40% – 60%: severe disability; 60% – 80%: crippled; 80% – 100%: bed-bound or exaggerating their symptoms).

Fluid content of the discs will be determined from T2-weighted sagittal images, and measured in the affected disc segment and in the contiguous 3 to 5 segments (Methods and Orozco et al). The fluid content values of the affected discs will e normalized to the values obtained from the healthy discs in the same individual (average value of the healthy discs). The change in fluid content is expressed as the ratio of fluif content at months 12 vs fluid content at baseline.

4.2 Secondary Outcome Measures:

Disability and quality of life evolution include Short Form (SF)-12 scores, global assessment by the patient and the physician. Overall pain intensity in the lumbar spine (1 = none, 2 = mild, 3 = moderate, 4 = severe, 5 = extreme); patient's global assessment of disease activity (1 = very good, 2 = good, 3 = fair, 4 = poor, 5 = very poor); physician's global assessment of disease activity (1 = very good, 2 = good, 3 = fair, 4 = poor, 5 = very poor) will be performed at 0, 3, 6, 12 and 24 months.

Additionally we will assess: de-/increase in rescue painkillers medication. Rescue medication use will be recorded throughout the study duration by a diary file. During the treatment phase of the study, a daily maximum of 3 g paracetamol/acetaminophen will be permitted. Opioid intake will be possible if paracetamol/acetaminophen is not sufficient. Only tramadol will be authorized.

Pain

The measurement of pain will be determined by self-report of current pain in rest and in motion in the low back pain using a VAS scale. In brief, the VAS consisted of a 10 cm horizontal line anchored with descriptors of pain including "no pain" on one end (left) and "extreme pain" on the other (right). After sitting for 10 minutes, subjects struck a vertical line through the 10 cm VAS representing their CP, and the distance from left end (no pain) to the vertical line was recorded in centimetres. In addition, the patients will fill VAS tests indicating the amount of pain experienced at rest, and in motion.

The drug consumption of painkillers will be assessed throughout the study at each visit. A diary file containing doses, drug name and indication will be given to each patient. The investigator will control this book at each visit. A reduction in dose or frequency of administration of painkillers is an indirect marker of the benefits of MSC therapy.

Employment and work status will be assessed. For this we will assign each of the patients to one of 4 categories designated as “employable” which included those who were unemployed due to pain, employed but on sick leave, laid off, or working. The other categories include retired, disabled, and elderly at least 60 years of age, eligible for social security.

Structural assessment: Evolution of affected disc(s) by quantitative Magnetic Resonance Imaging (MRI) density measurements in T2 and T1spin/echo and T1rho weighted images performed at 0, 6 12 and 24 months used as an indication of disc fluid and GAG content. The "quality" of the patient's lumbar disc will be monitored non invasively using T2-weighted MRI sagittal images (Orozco et al., 2011) (http://www.citospin.com/MESODISC/Fig_S3_.pdf) and, in T1spin/echo MRI. Lumbar disc grading will be performed in the sagittal T2 weighted images by two physicians independently who were experienced in MRI of the spine. They will review each intervertebral disc from L1–2 to L5-S1 by the modified Pfirrmann criteria. The modified Pfirrmann grading system assesses degenerated intervertebral discs by MRI for the asymmetry in disc structure, distinction of the nucleus and the annulus, signal intensity of intervertebral discs and height of intervertebral discs and assigns grade 1 to 8 for disc degeneration (Table by Griffin et al. Spine 2007).

Table 1. Modified Grading System for Lumbar Disc Degeneration*

Grade	Signal From Nucleus and Inner Fibers of Anulus	Distinction Between Inner and Outer Fibers of Anulus at Posterior Aspect of Disc	Height of Disc
1	Uniformly hyperintense, equal to CSF	Distinct	Normal
2	Hyperintense (>presacral fat and <CSF) ± hypointense intranuclear cleft	Distinct	Normal
3	Hyperintense though <presacral fat	Distinct	Normal
4	Mildly hyperintense (slightly >outer fibers of anulus)	Indistinct	Normal
5	Hypointense (= outer fibers of anulus)	Indistinct	Normal
6	Hypointense	Indistinct	<30% reduction in disc height
7	Hypointense	Indistinct	30%–60% reduction in disc height
8	Hypointense	Indistinct	>60% reduction in disc height

*Grades 1, 2, and 3 are based on the signal intensity of the nucleus and inner fibers of anulus. For Grade 4, the margins between the inner and other fibers of the anulus at the posterior margin of the disc are indistinct. For Grade 5, the disc is uniformly hypointense, although there is no loss of disc space height. For Grades, 6, 7, and 8, there is progressive loss of disc space height. These could be broadly classified as mild, moderate, to severe loss of disc space height. Very occasionally, although obvious disc collapse is present, hyperintense signal from the nucleus and inner fibers of the anulus is preserved. This is referred to by a double entry, *e.g.*, 4/7, with the former reporting the disc signal and the latter the degree of collapse.

Evaluation of cost:

We will compare the medical and non-medical costs between the two groups of patient. Costs will be identified for a one-year time horizon.

For this purpose, resource use in each arm will be collected in physical units in the eCRF at each clinical centre as follows:

- Acute care medical hospitalisations related to DDD
- Acute care surgical hospitalisations related to DDD
- Rehabilitation hospitalisations related to DDD
- Analgesics
- Work disruption

Resource use will be valued using production costs specific to each country or to the country having included the highest number of patients, depending on the number of patients actually included in each clinical centre.

Immune response / Analytical control

Although originally the beneficial effect of MSC was thought to be through engraftment and regeneration, subsequent studies demonstrated that the main therapeutic effects may be mediated primarily through the secretion of soluble factors and their impact on immune cells. Indeed, MSC have been shown to (1) inhibit the differentiation and activation of T cells (2) promote induction of immunosuppressive FoxP3+ T-regulatory cells, (3) induce Breg cells (4) impact DC and macrophage differentiation. In this project, the use of allogeneic MSC and their putative impact on immune cells sustains the need to monitor the recipient immune response. The assessment of the biological effect of allogeneic MSC on recipient immune response will be studied by multiparametric flow-cytometry as well as monitoring of anti HLA-I antibodies response. As nucleus pulposus (NP) is an immuno privileged tissues, without vascularisation, so we do not expect cell rejection. However, selection of patient according to the presence of initial antiHLA Ab in the serum is critical as well as follow up of allo response in case we will need further cell injection in the future. We will perform the HLA-genotyping of MSCs and will assess MSC immunogenicity investigating the emergence of anti-HLA class 1 antibodies in MSC-injected patients before and after treatment.

This large-scale immunomonitoring will be possible on the two standardized platforms of the ECELLFRANCE infrastructure (the first located in Rennes under the supervision of Pr Karin Tarte and the second located in Montpellier, Pascale Louis-Plence). The two platforms have been already involved in the immunomonitoring of the MSC-recipients in previous phase 1 clinical trials (ADIPOA 1, SCLERODERMIA) and will be involved in up-coming phase 2 clinical trial (ADIPOA 2, RESSTORE). Results from the phase 1 clinical trial ADIPOA 1, showed a significant increase in the frequency of Tregs and transitional B cells as well as a decrease of classical inflammatory monocytes at 3 month following MSC injection, underscoring the immunomodulatory properties of MSC. Anti-HLA antibody testing provides an accurate assessment of an individual sensitization status following allogeneic cell transplantation. Thus, the presence of anti-HLA Class I antibodies will be assessed systematically in all 112 patients using Flow-Cytometry bead-based assays (Luminex™) before and after MSC injection. In case of positive detection (evaluated at 30% of the MSC-treated patients), HLA class I typing will be performed by sequencing as well as identification of the HLA antigens specifically targeted by those antibodies by Luminex technology.

Safety Outcomes

Prior to enrolment into the trial, subjects will undergo a thorough screening to assess their eligibility for the study, including X-ray and MRI of the lumbar spine, recording of medical history, concomitant medications, vital signs, physical examination, pregnancy test, routine laboratory testing, urinalysis and serological test of infections.

The results from the above mentioned examinations and tests will be used as baseline values and comparators, if and as far as changes of these parameters will occur during the trial.

During each visit to the study centre, participants will undergo a physical examination with recording of vital signs, blood sampling for assessment of routine lab tests, urinalysis, a spine examination and an assessment of pain. Furthermore, if the study patients do not spontaneously report any AE occurrence since their last visit, they will be interviewed by the investigator filling a study-specific AE checklist for recording of symptoms and complaints.

If an AE occurs at a moment in-between two study centre visits and participants feel that they should consult a physician, they have to call or visit the emergency service of reanimation referring to their participation in the study.

5 Investigational Investigational Medicinal Product (IMP) and reference arm

5.1 Presentation of the Investigational Medicinal Product

The IMP is composed of allogeneic bone marrow MSCs suspended in HypoThermosol^{R} . It is formulated as a cellular suspension for intradiscal use in syringe ready to use.

The doses have been defined in previous phase 1 and 2a clinical trials: 20 x 10^6 MSC±10% in 2 ml (cell concentration 10x10^6/mL) with good results.

The cell suspension is packaged in a EC marked syringe sealed with a sterile luer lock cap and placed in a sterile, sealable (with inviolable sticker) box for delivery to the clinical sites under hypothermic conditions.

In the control arm, the patient is subjected to the usual physical therapy, postural hygiene and pharmacotherapy. In addition, a sham-maneuver as in the cell-treated patients are added, consisting in anesthetic infiltration with 2 ml of 1% Xylocaïne in the paravertebral muscles close to the affected segment.

In the experimental arms the IMP are allogeneic BM-MSCs processed according to the authorized procedure (MSV; IMP 15-007, which has been improved for the RESPINE assay; see IMPD for details). Cell dose will be 20±5 million cells suspended in 2 ml of HypoThermosol isotonic transport solution), were the cells are viable for up to 72 hours at 2-8 ºC. This time will be sufficient for transport to the different application hospitals. The whole process for obtaining the MSV (for *Valladolid* MSC) is carried out by highly qualified cell culture specialists at the Cell Production Unit of Citospin (Valladolid University Scientific Park), which fulfills the GMP criteria and has been approved for manufacturing, handling and distributing both autologous and allogeneic BM-MSCs by the NCA, the Spanish Medicine Agency.

In validations performed previously, it has been shown that MSV cells possess the typical characteristics of MSC defined by the Society for Cellular Therapy:

1) Adhesion to plastic in culture.

2) Expression of antigens CD73 CD90 and CD105 (Dominici et al, 2006).

3) Absence of hematopoietic antigens, markers of monocytes, macrophages and lymphocytes B. Details:http://www.citospin.com/MESODISC/Fig_S1_.pdf and http://www.citospin.com/MESODISC/Fig_S2_.pdf;

4) *In vitro* differentiation capacity into chondrocytes (Orozco et al, 2011).

5.2 Obtaining, purifying and expanding MSC.

Processing and expansion of the collected BM-MSCs will be done using the NCA-authorized Citospin normalized procedure http://www.citospin.com/MESODISC/EudraCT_2012-004444-30.pdf) during 3-4 weeks. The responsible technical team is expert in handling, expansion and selection of MSC. For the shake of homogeneity cells will always be manufactured by Citospin at Valladolid. A potential improvement of the protocol will be validated and investigated during the first 6-12 months, and embraced if results are positive, It consists of cell expansion by the usual protocol during 3 weeks followed by cryopreservation of the cellular stock. At the adequate moment revitalization and transport to the clinical units under hypothermic conditions (described above) will be performed. This would permit a better logistics of both cell production and clinical application and, more important, would eventually allow study of the different cell batches before infusion.

The source of cells for obtaining MSV is bone marrow aspirate from healthy donors undergoing a normalized anticoagulation protocol. Upon receipt of the bone marrow sample (in a period shorter than 24 hours), and after checking perfect shipping status, presence of serum sample for PCR/NAT and correctness of the accompanying documentation (informed consent of donation form, serology, haemogram), the sample will processed by Citospin at the University of Valladolid Scientific Park facilities. In essence, the mononuclear cells are separated from the bone marrow aspirates by Ficoll density gradient and a study of cellular viability is carried out. During culture and expansion, cells will be subjected to two trypsinization/re-seeding processes (passes), which aim to multiply and purify the mesenchymal cell line. Culture extends for a long period, 3-4 weeks, until enough cells have been produced. The detailed specifications are contained in the IMPD and in the Production Guide entitled “Development of Cell Therapy: expansion of mesenchymal cells from bone marrow: (FA-CMES - 001-P)”. Donor serum samples are also obtained to carry out the required serum tests for excluding VIH and hepatitis contamination (RD 1301 to 2006 for human tissues and cells, see IMPD). The tests must be performed using PCR (NAT, Nucleic Acid Technology) to circumvent quarantine (Roth, 2010). A part of the sample must be kept to allow further analysis if required. HLA typing I and II and mixed lymphocyte reaction (MLR) will also be performed.

At the end of the expansion process, routine immunophenotypic characterization of the resultant MSV is performed by flow cytometry, for the established MSCs markers. The phenotype must be positive (=> 95 %) for CD73, CD105 , CD90 , CD166 and negative (=<10%) for CD45, CD14, CD34, and HLA –DR. Then the cellular stock is cryopreserved and stored in liquid nitrogen until the cells are required. At this time the cellular stock is subjected to revitalization. Finally, cells are harvested, suspended in Hypothermosol at 10 millions/mL and packed in 5 ml syringes suspended in 20 million cells in 2 mL of hypothermic transport solution (Hypothermosol). Sterility and cleanliness conditions, as well as quality control inside the Cell Production Unit will be maintained by following the steps detailed in the “Normalized Operating Procedures GMP normative”.

5.3 Methodology for obtaining bone marrow from donors.

The methodological protocol is described in EudraCT 2005-001755-38 (see details in http://www.citospin.com/MESODISC/NCT01860417long.pdf). Briefly, puncture and bone

marrow aspiration will be performed in an ambulatory surgery setting. The patient, in prone position, accommodated with pillows, will undergo light sedation. The surgical field is brushed with alcoholic povidone-iodine solution (chlorhexidine if a history of allergy to iodine exists) and sterile fields delimitation, leaving both posterior iliac crests free. After local anesthesia (10 cc + 10 cc of 1% lidocaine without epinephrine), two members of the extractor team, placed on both sides of the operating table, will perform several punctures with a 11-G trocar under the iliac spine, aiming toward the posterior sacroiliac joint (this is the iliac area with higher trabecular density). The technique involves cortical perforation and repeated sudden aspiration of small bone marrow volumes (2mL maximum) to minimize contamination with the peripheral blood. The aspirate is injected into a heparinized bag provided for transport. Two successive aspirations are performed by rotating 90 degrees clockwise the beveled trocar. The same puncture hole allows a further 1-2 mm deepening twice, repeating the same methodology with 1 mL suction, syringe change, 90º bezel rotation and new aspiration, continuing on both sides of the pelvis until about 80 mL are collected. Bone marrow (sterile bag heparinized with a volume of about 80 mL of aspirate) will be refrigerated to 4ºC, conditioned and shipped to the Cell Therapy Unit. Further processing should be done before 24 h. Briefly, mononuclear cells from 80-100 ml of bone marrow aspirate are isolated and selected by adherence to plastic in T-175 flasks. Depending on the performance, which is very variable from patient to patient, we obtain at this stage between 6 and 45 millions of adherent cells, which further expand about 10 times in the following 3 weeks for use in the clinical trial. Since the required number of MSC will be 20 or, at most, 40 million cells per patient, an important surplus of cells can be obtained in many cases, which should be enough for 5 or more allogeneic patients.

5.4 Selection of donors of mesenchymal cells for treatment of the group receiving allogeneic cells.

Bone marrow will be obtained from donors after informed consent signature.

Donors must meet all the criteria established for selection of blood donors and donors of hematopoietic precursors (RD 1088/2005;of September 16 stating technical requirements and minimum conditions for blood donation and centers and services for transfusion).

• Must sign the informed consent form (ICF).

• Must undergo serology for HIV, hepatitis B and C, Syphilis using PCR test (NAT technology to avoid window effect) and provide a serum sample for serum bank

5.5 Packaging and conditioning for shipping of final product.

MSV are suspended in 2 ml IBTS and charged in a 5 ml syringe labeled with the name and lot number of the product. The product can be stored for 48 hours at 2-10 °C according to our protocol. The drug is transported in coolers validated for such activity, keeping it between 2 and 10°C. It has been found that, under these conditions, viability is preserved for at least 24 hours (Orozco et al., 2011).

5.6 IMP labelling, storage and handling

MSV are labeled by the manufacturer according to the applicable regulatory requirements ensuring the traceability and attribution to the individual recipient of the MSV. A specimen

label is provided in the IMPD

MSV will be received at each hospital pharmacy, where a quality control will be conducted. The pharmacist will control if the storage condition during the shipment (temperature between 2 and 10°C) has been respected, the compliance of the pharmaceutical product with delivery note (labelling, batch number, date of expiration)

When received, The IMPs will be stored in the hospital pharmacy between 2° and 8 °C until dispensation to care units.

The IMPs/placebo will be provided to the care unit in charge of the administration of the product to the patient in accordance with the investigator's prescription. The batch number and expiry date will be mentioned on the prescription.

A traceability system will be implemented by hospital pharmacies. Information such as MSV administration and batch release will be registered. Information such as patient code, batch number, expiry date and also date/hour of dispensation will be recorded. The traceability system will also involve a nominative accountability of delivered MSV.

The whole pharmaceutical cirtcuit will be detailed in a specific SOP provided by the sponsor

5.7 Application of the cells.

Implantation of the cells is performed under light sedation and fluoroscopy control. The cells are injected by disc puncture avoiding neurovascular elements (Konings & Veldhuizen, 1988). After regional double brushing with aqueous povidone-iodine solution (chlorhexidine or iodine in allergic patients), the field is delimited with sterile sheets and local anesthesia (1% Xylocaïne) is applied to the skin, the subcutaneous tissue and muscles close to the puncture site. With fluoroscopy in antero-posterior position, a vertical line corresponding to the projection of the spinous processes is marked on the skin with a sterile dermographic pencil. Then, a perpendicular line corresponding to the projected Kirschner needle aligned with the intervertebral space to be treated is drawn, and a 22G spinal needle is inserted at an angle of 25-35 degrees towards the midline at a point located 8 to 9 cm (depending on patient morphotype) from the midline. At this point, fluoroscopy is changed to the lateral position to ensure that the penetration of the needle is in the right direction, and the needle is pushed until it reaches the nucleus pulposus. After verifying the correct position of the needle both in the anterior-posterior and the lateral fluoroscopic view the cell suspension is injected slowly according to Meisel disc cells injection procedure. By using this procedure, we had no incidents neither at the clinical trials (Orozco et al., 2011; Noriega et al., Aug;101(8):1945-1951.Transplantation 2017) nor at any of the additional 170 patients previously treated in compassionate use.

5.8 Application of sham procedure

In the other group, the sham procedure will be performed in the same conditions in order to keep blind the patient. The radiologist in charge of injection, who's the only unblinded investigator will mimic intradiscal injection by using imaging with only paravertebral muscle puncture. No local saline injection has been planned.

the study will be blinded for patient and for the investigator in charge of patient inclusion and follow-up the physician that performs the injection will keep the patient blinded.

5.9 Post-treatment care

The patient is discharged after an observation period of 2 hours. Walk for moderate periods is authorized. Labor activity is suspended for one week. The pain medication is tailored to the needs of each patient and the use of NSAIDs should be avoided. The long-term monitoring will consist of out-patient visits and monitor- radiographic and MRI

The patients are followed for 2 years on clinical examination , spine MRI and immune response. For 5 years they will be followed annually on clinical basis to assess any unexpected long term side effect.

In case the patient have not responded after end of study (2 years) or relapse, he may be eligible for cell therapy when the product will be available on routine basis, or benefit for treatment recommended by EULAR (physiotherapy, steroid or surgery)

5.10 Possible complications of this therapy.

The complications are the same as in the discography, and rarely presented. The most common complaint is the exacerbation of pain for 1-2 weeks, which is usually solved by analgesia and muscle relaxants for a short period. The most serious complication is discitis, occurring in less than 0.1 % (Osti et al., 1990). Other complications described, although much less frequently, are transient headache, nausea, meningitis, epidural abscess, arachnoiditis, intrathecal hematoma, retroperitoneal hematoma, cauda equina syndrome and acute disc herniation. Cases of urticaria are not likely in this therapy, as they are attributable to the radiologic contrast, which is not used here. Initial fears about the possible long term side effects of discography regarding the disc viability have not been confirmed. A 20-year of clinical follow-up did not find radiographic evidence of progressive disc degeneration after the discographies (Flanagan & Chung , 1986).

The possible risk of complications from the use of allogeneic cells has been discussed in detail above. The summary is that, according to prior experiments reported in the literature, the application of allogeneic MSC, both locally and systemically, are safe as we have used the cell product in previous study without significant reported SAE. We do not expect ectopic tissue formation, as this has never been observed with MSC compared to iPS. Teratoma are obtained after iPS reprogrammation, as cell acquires multipotenty. This is not the case of MSC, as they are tripotent, with possibility to differentiated to adipose osteoblast or chondroblast. MSC have been studied in 400 studies including thousands of patients ; ref Yubo M, Yanyan L, Li L, Tao S, Bo L, Lin C (2017) Clinical efficacy and safety of mesenchymal stem cell transplantation for osteoarthritis treatment: A meta-analysis. PLoS ONE 12(4): e0175449. https://doi.org/10.1371/ journal.pone.0175449

In the present trial, the reactivity of the skin and mucosa will be surveyed in order to monitor a possible immunological reaction.

5.11 Risks of graft rejection

Allogeneic MSC are immunoelusive (Ankrum et al. 2014) and are exceptionally well tolerated by the host immune sytem. However, a risk of graft rejection can not be totally ruled out. Thus, the signs of a graft rejection reaction will be monitored including skin rash

local inflamations. Only 3 patients out of previous study developed anti-HLA to allogenic cells but no correlation with response was observed.

We will assess 6 months after the injection of cells emergence of Anti-HLA ab.

6. Study procedures and visits

Each person must be fully informed prior to being enrolled to the clinical trial. Eligible subjects have to be informed in person by the investigator and in writing by the patient information. Only after clarification of all questions this person is asked to sign two copies of the consent form and date by hand. Then a copy of patient information and informed consent of the eligible person shall be issued; the second copy of the consent form is kept in the ISF.

6.1 Schedule of assessment

CHRONOGRAM	Pre-screening	Screening	Inclusion/randomization	Baseline Treatment	Tracking (M = months)				
PROGRAMMING OF VISITS:	**Day -47/-50**	**Day -40/-21**	**Day -21/-15**	**V0 Day 0**	**V1 M1 (+/-7)**	**V2 M3 (+/-14)**	**V3 M6 (+/-14)**	**V4 M12 (+/-30)**	**V5 M24 (+/- 30)**
Instructions for wash-out 48 hours NSAIDs[a] washout	X	X	X	X					
Prohibition of NSAIDs[a]				X	X	X	X		
Instructions for wash-out 24 hours painkillers[b] washout	X	X	X	X	X	X	X	X	X
Delivery of the Information sheet	X								
Signature of Informed Consent form	X								
General medical History and Demography		X							
Lumbar related medical history		X							
Vital signs [c]		X	X	X	X	X	X	X	X
Physical Exploration		X	X	X	X	X	X	X	X
RX of Lumbar Spine Simple and Dynamic		X							X
ECG 12-Lead		X							

CHRONOGRAM	Pre-screening	Screening	Inclusion/randomization	Baseline Treatment	Tracking (M = months)				
PROGRAMMING OF VISITS:	**Day -47/-50**	**Day -40/-21**	**Day -21/-15**	**V0 Day 0**	**V1 M1 (+/-7)**	**V2 M3 (+/-14)**	**V3 M6 (+/-14)**	**V4 M12 (+/-30)**	**V5 M24 (+/- 30)**
Blood sampling for routine laboratory testing and urine analysis		X	X	X	X	X	X	X	X
Urine pregnancy test		X	X	X					
Preceptive Viral Serologies		X							
Checking Inclusion and exclusion criteria		X	X						
Randomization			X						
Serology anti-HLA				X			X		
Optional Immunomonitoring and miRNA			X[d]	X[d]	X		X		
Pain lumbar spine VAS, Oswestry, SF-36		X	X	X	X	X	X	X	X
Lateral MRI, T2, quantitative		X[e]					X	X	X
Intervention: Control or Allogeneic cells****				X[f]					
Adverse Events				X	X	X	X	X	X
Concomitant medication		X	X	X	X	X	X	X	X

[a] NSAIDs washout of at least 2 days before screening, inclusion and baseline. NSAIDs will not be authorized during the first 6-month after baseline . However, if needed after the first 6 months, NSAIDs could be used
[b] Patients will be required to refrain from taking paracetamol or opioids within 24 hours of each clinical visit for efficacy evaluations
[c] vital signs: Temperature, heart rate, arterial blood pressure, height, weight
[d] will be done at inclusion OR V0
[e] MRI was also used to assess the Pfirrmann's score
[f] RX in the operating room to guide and monitor cell implantation

6.2 Visits during the course of the trial

6.2.1 Pre-Screening visit (Day-50 ±47)

- Delivery of the Information sheet and signature of the consent form before any procedure related to the study
- **Instruction for wash out (NSAIDS washout of at least 2 days before the screening Visit, no painkillers 24h before the screening visit) will be given to the patient after written informed consent.**

6.2.2 Screening visit (Day-40 ±21)

- Demografics and general medical history.
- Lumbar related medical history (includes past history of osteoarthris, osteoporosis, lumbar pain, injury, trauma, fractures, chirurgical procedure or other conditions.
- Medication history therapy and unresponsive (including physical therapy) for at least 3 months.
- Vital signs after sitting for at least 5 minutes, (temperature, blood pressure, and heart rate, height, weight)
- Musculoskeletal physical examination
- Lumbar spine examination (See Appendix 1):
- Pain assessment of the lumbar spine by visual pain scale (VASOswestry Disability Index in order to measure a patient's permanent functional disability, Quality of life assessment using SF-36
- X-rays of lumbar spine simple and dynamic
- If clinical and radiological criteria fulfilled, perform ECG (12-lead)
- Blood sampling for routine laboratory testing

  Haematology: haematocrit, haemoglobin, RBC, WBC; PC

  Biochemistry: Electrolytes, Creatinine, BUN, ALT, AST, AP, Bilirubin, ,

  Coagulation (INR, PTT)

  CRP

- Urinalysis (dipstick): Glucose, Ketones, blood, protein, nitrite, leucocytes, , bilirubin in urine

  Urine pregnancy test in woman of childbearing potential

- Blood sampling for preceptive viral serologies **:**

  HBs antigens

  Anti-HBc antibodies

  Anti-HCV antibodies

  Anti-HIV-1 and anti-HIV-2 antibodies,

  Anti-HTLV-1, HTLV-2 antibodies

Wasserman testing

- Blood testing for Syphilis serology

6.2.3 Inclusion visit and randomization (day -21/-15)

- Checking and confirm inclusion and exclusion criteria
- General physical examination including height, body weight, BP and HR
- Musculoskeletal physical examination
- Lumbar spine examination as well as Lasegne testing (See Annexe III):
- Medication history therapy
- Pain assessment of the lumbar spine by visual pain scale (VASOswestry Disability Index in order to measure a patient's permanent functional disability, Quality of life assessment using SF-36
- Blood sampling for routine laboratory testing

  Haematology: haematocrit, haemoglobin, RBC, WBC; PC

  Biochemistry: Electrolytes, Creatinine, BUN, ALT, AST, AP, Bilirubin, ,

  CRP
- Urinalysis (dipstick): Glucose, Ketones, blood, protein, nitrite, leucocytes, , bilirubin in urine
- Urine pregnancy test in woman of childbearing potential
- Blood sampling for Immunomonitoring (37.5 ml) and for miRNA (10 ml)
- Instruction for wash out (NSAIDS washout of at least 2 days before this screening Visit, no painkillers 24h before the next visit V0, V1, V2) and instructed not to use NSAIDS at all visits up to 6 months after inclusion.
- Randomization

Randomization procedure: the randomization is performed 24 hour-a-day online by the central randomization web-service customized by the **IZKS Mainz**. To randomize a patient, investigators have to login, enter the patient identification, additional checks and factor values. The radiologist in charge of cell injections will be strictly forbidden from discussing treatment allocation with patients and clinical observers.

6.2.4 Day 0 - Visit 0 :Visit baseline and treatment

- General physical examination including height, body weight, BP and HR
- Musculoskeletal physical examination
- Lumbar spine examination (See Annexe 1):
- Medication history therapy
- Blood sampling for routine laboratory testing

  Haematology: haematocrit, haemoglobin, RBC, WBC; PC

Biochemistry: Electrolytes, Creatinine, BUN, ALT, AST, AP, Bilirubin, ,
CRP

- Urinalysis (dipstick): Glucose, Ketones, blood, protein, nitrite, leucocytes, bilirubin in urine
- Urine pregnancy test in woman of childbearing potential
- Serology anti-HLA
- Blood sampling for Immunomonitoring (37.5 ml) and for miRNA (10 ml) ***only if not done at the inclusion visit***
- Pain assessment of the lumbar spine by visual pain scale (VAS)
- Oswestry Disability Index in order to measure a patient's permanent functional disability, Quality of life assessment using SF-36
- Instruction for wash out (NSAIDS washout of at least 2 days before this screening Visit, no painkillers 24h before the visit)
- Adverse event assessment
- Intervention : injection of allogenic cells or sham procedure (under RX to guide and monitor cell implantation)

6.2.5 Visit 1: Month 1

- General physical examination including height, body weight, BP and HR
- Musculoskeletal physical examination
- Lumbar spine examination as well as Lasegne testing (See Annexe 1):
- Blood sampling for routine laboratory testing

  Haematology: haematocrit, haemoglobin, RBC, WBC; PC

  Biochemistry: Electrolytes, Creatinine, BUN, ALT, AST, AP, Bilirubin,

  CRP
- Blood sampling for Immunomonitoring (37.5 ml) and for miRNA (10 ml)
- Urinalysis (dipstick): Glucose, Ketones, blood, protein, nitrite, leucocytes, urobilinogen, bilirubin in urine
- Pain assessment of the lumbar spine by visual pain scale (VAS)
- Oswestry Disability Index in order to measure a patient's permanent functional disability, Quality of life assessment using SF-36
- Adverse event assessment
- Instruction for wash out (NSAIDS washout of at least 2 days before this screening Visit, no painkillers 24h before the visit)

6.2.6 Visit 2: Month 3

- General physical examination including height, body weight, BP and HR

- Musculoskeletal physical examination
- Lumbar spine examination (See Annexe 1):
- Blood sampling for routine laboratory testing

  Haematology: haematocrit, haemoglobin, RBC, WBC; PC

  Biochemistry: Electrolytes, Creatinine, BUN, ALT, AST, AP, Bilirubin,

  CRP
- Urinalysis (dipstick): Glucose, Ketones, blood, protein, nitrite, leucocytes, , bilirubin in urine
- Pain assessment of the lumbar spine by visual pain scale (VAS)
- Oswestry Disability Index in order to measure a patient's permanent functional disability, Quality of life assessment using SF-36
- Adverse event assessment
- Instruction for wash out (NSAIDS washout of at least 2 days before this screening Visit, no painkillers 24h before the visit)

6.2.7 Visit 3 : Month 6

- General physical examination including height, body weight, BP and HR
- Musculoskeletal physical examination
- Lumbar spine examination (See Annexe 3):
- Blood sampling for routine laboratory testing

  Haematology: haematocrit, haemoglobin, RBC, WBC; PC

  Biochemistry: Electrolytes, Creatinine, BUN, ALT, AST, AP, Bilirubin,

  CRP
- Serology antiHLA
- Blood sampling for Immunomonitoring (37.5 ml) and for miRNA (10 ml)
- Urinalysis (dipstick): Glucose, Ketones, blood, protein, nitrite, leucocytes, urobilinogen, bilirubin in urine
- Lateral MRI, T2, quantitative
- Pain assessment of the lumbar spine by visual pain scale (VAS)
- Oswestry Disability Index in order to measure a patient's permanent functional disability, Quality of life assessment using SF-36
- Adverse event assessment
- Instruction for wash out (No painkillers 24h before the visit)

6.2.8 Visit 4 : Month 12

- General physical examination including height, body weight, BP and HR
- Musculoskeletal physical examination

- Lumbar spine examination (See Annexe 3):
- Lateral MRI, T2, quantitative
- Pain assessment of the lumbar spine by visual pain scale (VAS)
- Oswestry Disability Index in order to measure a patient's permanent functional disability, Quality of life assessment using SF-36
- Blood sampling for routine laboratory testing

  Haematology: haematocrit, haemoglobin, RBC, WBC; PC

  Biochemistry: Electrolytes, Creatinine, BUN, ALT, AST, AP, Bilirubin, , CRP,
- Urinalysis (dipstick): Glucose, Ketones, blood, protein, nitrite, leucocytes, , bilirubin in urine
-
- Pain assessment of the lombar spine by visual pain scale (VAS) (See Annexe)
- Adverse event assessment
- Instruction for wash out (No painkillers 24h before the visit)

6.2.9 Visit 5 : Month 24

- General physical examination including height, body weight, BP and HR
- Musculoskeletal physical examination
- Lumbar spine examination (See Annexe 3):
- Lateral MRI, T2, quantitative
- Pain assessment of the lumbar spine by visual pain scale (VAS)
- Oswestry Disability Index in order to measure a patient's permanent functional disability, Quality of life assessment using SF-36
- Blood sampling for routine laboratory testing

  Haematology: haematocrit, haemoglobin, RBC, WBC; PC

  Biochemistry: Electrolytes, Creatinine, BUN, ALT, AST, AP, Bilirubin, , CRP,
- Urinalysis (dipstick): Glucose, Ketones, blood, protein, nitrite, leucocytes, urobilinogen, bilirubin in urine
- X-rays of lumbar spine simple and dynamic
- Pain assessment of the lombar spine by visual pain scale (VAS) (See Annexe)
- Adverse event assessment
- Instruction for wash out (No painkillers 24h before the visit)

6.3 Unscheduled visits

If subject gets in contact with the site between regular visits due to acute disorders, an unscheduled visit will promptly be performed at the site. During this additional visit a physical examination, a systematic interview for recording AEs should be performed. In

case of suspected local infection (nocturn lumbar pain, temperature above 38°C), disc puncture will be performed and an empirical antibiotic treatment will be initiated immediately and further tuned according to the antibiogram

6.4 Concomitant therapy and Rescue therapy:

Rehabilitation: Lifestyle modification, particularly exercise and weight reduction, is a core component of the management of low back pain. Each investigator will be free to prescribe the rehabilitation that will be standardized through SOP included in annex. Indeed, it will be recorded as a rescue medication.

Rescue medication use will be recorded for the study duration. Within 24 hours following MSC injection, paracetamol/acetaminophen will be advised for each patient if needed.

Patients will be required to refrain from taking paracetamol or opioids within 24 hours of each clinical visit for efficacy evaluations. If the patient required, supplementary analgesic medication will be providedTramadol will be used as rescue medication.

NSAIDs will not be authorized during the first 6-month of the study. However, if needed after the first 6 months, NSAIDs could be used.

A diary file will be provided for each patient in order to collect information on medications use, mobility, activities and rehabilitation sessions.

7. Biological sampling and collection

Blood samples, RNA, will be taken in each centre and stored if necessary. To summarize:

- Blood sample (10 ml) will be used to analyse inflammatory markers and assess the the effect of MSC before and after cell injection. The time points are V0 (Baseline), Month 3 and month 6. The samples will be analysed at Inserm U1183
Institut de Médecine Régénératrice et Biothérapies (IRMB) Hôpital Saint-Eloi, Montpellier, France (under the supervision of Dr Danièle Noël)

- Another blood sampling (37.5 mL) will be used to assess the biological effect of the MSC injection on recipient immune response. Three time points have been selected, the day of MSC-injection for the baseline and 1 and 6 months following MSC-injection. We will assess the T cell subpopulation as well as the monocyte subpopulation. We have previously shown that MSC administration may impact the immune system and modify lymphocytes and monocytes population. We will assess increase in foxP3, CD4, CD49b CD25 Tcells and decrease in transitional CD38 B cell population, as well as a decrease in CD14 CD16 circulating inflammatory monocytes. These markers will be assessed by FACS analysis in the validated immunomonitoring platform before and 6 months after cell therapy. These monitoring is optional and will be performed on 60 patients from 4-5 centres. These samples will be sent to the Ecellfrance immunomonitoring platforms within 24 hours. Actually, two platform exist and will receive fresh blood samples (IRMB, INSERM U1183, Saint-Eloi Hospital, Montpellier (under supervision of Dr Pascale PLENCE) and INSERM U917, Rennes 1 University, Rennes, France (under supervision of Pr Karin TARTE). These two platforms will perform a large scale immunomonitoring to assess the frequency and the activation status of several immune cells. They will store the biological samples (plasma if available). T

- Finally, Blood sample (10 ml) will be used to perform the HLA-genotyping of MSCs and will assess MSC immunogenicity investigating the emergence of anti-HLA class 1 antibodies in MSC-injected patients before and after treatment. The Analysis of the immunogenicity will be performed by a central laboratory in collaboration with Ecellfrance immunomonitoring platforms and the results will be reported as specific adverse reaction related to the treatment .

At the end of the study, residual samples from the analysis mentioned above will be stored at IRMB, Saint-Eloi Hospital, 295 avenue Augustin Fliche, 34295 Montpellier, FRANCE, under the supervision of Pr Christian JORGENSEN. These samples could be used for scientist research related to the Evaluation of biological parameters in blood.The samples will be stored 10 years maximum. Each sample will be stored pseudomysed. The consent of the patients will be required for this storage.

Within the framework of the Respine European project, samples from donors will be transferred and analysed in differents institutions of the consortium. The consent for storage and analyses of donors samples must be obtained from donors according to local laws. Sample transfert from an institution to another must be recorded in a material trasfert agreement according to the consortium agreement,

The Appendix 2 descibes for the whole biological sampling

8. Statistic analysis planning and power calculation

The study is designed to conclude with a Type 1 error of 5%, and a Type 2 error between 10 and 19%. We will include a total of 112 patients, 56 patients in the control group (sham injection), 56 patients in the treatment allogeneic group (allo BM-MSCs).

According to the literature data (Manchikanti L et al, Pain Physician 2014; 17:E61-E74, Mesoblast trial, our own preliminary data in EudraCT 2012-004444-30) a responder rate (improvement VAS or ODI score of at least 20%) is expected at 12 months in 30% in controls and 60% for BM-MSC, i.e. a delta of 30% between the two groups. To highlight this difference while justifying a power of 90% and an alpha risk of 5% (bilateral hypothesis) and within 1-1 ratio, taking into account 10% of inclusion failure, it is necessary to include 56 individuals per group for a total of 112 subjects.

Besides, after Noriega's data, we expect a disc density increase of 22% in cell-treated discs, and 6% in controls, with a standard deviation of 11%. To highlight this difference while justifying a power of 90% and an alpha risk of 5% (bilateral hypothesis) and within 1-1 ratio, taking into account 10% of inclusion failure, it is necessary to include 14 subjects per group.

As these two outcomes are co-primary endpoints, the power of the study will depend on their association (Multiple Endpoints in Clinical Trials, Guidance for Industry. FDA 2017) : if they are independant, the power will be of 81%, and if they are completely dependant, the power will be of 90%.

8.1 Statistic analysis

A description of statistical methods follows and will be described in more details in the Statistical Analysis Plan (SAP). All statistical analyses will be performed using SAS® (SAS Institute, Cary, NC, USA).

All confirmatory statistical tests will be analyzed using 2-sided significance tests at the 0.05 alpha level. P-values will be presented to 3 decimal places.

8.2 Primary efficacy analysis

The main analysis will be in intent-to-treat (Randomized Set), including all the randomized patients after multiple imputation of missing data.

The responders' rate after 12 months of treatment will be compared between groups with chi-square test. The relative risk (RR) and its 95% confidence limits will be reported to estimate the effect size.

The mean change of fluid content of affected disc will be compared between groups with a Student's-test

8.3 Sensitivity analysis

Sensitivity analysis will be performed in the full data set population (including all subjects who are randomized, receive the injection, and have a valid primary efficacy baseline measurement and at least one valid post-baseline primary efficacy measurement). We will also test a worst-case maximum bias scenario, in which we assume that all patients with missing data are not responders in the intervention group and responders in placebo group.

. Multivariates regression will be used to test the impact of covariates not comparable at baseline, and to test the impact of a rehabilitation program attended during the follow-up.

8.4 Analysis of the secondary outcomes

The change from baseline to the 3, 6, 12 and 24 months values on secondary outcomes (VAS, ODI, SF-36, structural assessment, immune response) will be analysed with mixed models. Estimate of treatment effects for the difference MSC-placebo and their 95% confidence intervals will be reported.

Employment and work status at months 12 and 24 will be compared between groups with chi-square test. The corresponding RR and its 95% confidence limits will be reported.

Costs at month 24 will be compared between groups using parametric and non-parametric tests.

8.5 Safety analysis

Adverse events that occur during this study will be presented by system organ class (SOC), high level term (HLT), and preferred term (PT) in a frequency table giving the number of events, the number of subjects, and the percentage of subjects who experience the event by treatment group.

Subjects with multiple AEs will be counted only once within each PT, HLT, and SOC. Coding of the AEs will be performed with the Medical Dictionary for Regulatory Activities.

8.6 Handling of protocol deviations

The clinical project manager will prepare the Specification of Protocol Deviations in consultation with the Clinical Trial Biostatistician and Study Physician. Search criteria for potential protocol deviations will be detailed in the Specification of Protocol Deviations. During the blind period, the protocol deviations will be classified according to their impact on the primary objective.

All important protocol deviations, including missing relevant data, will be listed by subject.

8.7 Handling of dropouts or missing data

Rules for imputation of missing data will be detailed in the SAP. When analyzing efficacy data, if the missing data is type MAR (Missing At Random) or MCAR (Missing Completely At Random), a multiple imputation will be implemented.

9. Assessment of Safety and Tolerability :

Safety and tolerability will be evaluated by recording adverse events (AEs) and serious AEs (SAEs) throughout the study, on all patients enrolled in the study. Number of participants with adverse events, type of adverse event and their repartition between treatment arms will be used as a measure of safety and tolerability.

9.1 Safety parameters :

The following parameters will be assessed and analysed for study product safety :

- clinical review and questionnaires for pain,
- disability and quality of life at 0, 3, 6 12 and 24 months
- physical examinations performed at baseline and week 1, M3, M6, M12 and M24 after injection of the cells;
- laboratory tests (haematology, blood chemistry) and vital signs assessed at baseline and week 1, M6, M12 and M24. Elevations in ALT and/or AST >3 × ULN will also be reported since this level was considered to be most relevant to health authorities and healthcare providers.

9.2 Definition

Adverse Event :

An adverse event is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An adverse event, whether or not considered to be the causally related to the investigational medicinal product, may be:

The deterioration of a pre-existing chronic disease or aggravation of a symptom or disease that was present on enrolment of the patient in the study

A symptom or disease discovered after the start of the study even if it was probably present prior to the patient's enrolment in the study.

Abnormal laboratory findings will be considered as adverse event when they are considered /judged as clinically significant by the investigator.

Clinically significant event/value should be defined as: symptomatic, requiring corrective treatment, leading to discontinuation, dose delay, dose reduction, dose interruption and/or fulfilling seriousness criteria.

Adverse drug reaction (ADR)

All noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions.

An adverse reaction is an AE that is at least possibly related to the administration of the study medication or procedure. When assessing the relationship (“related” or “unrelated”) between an AE and the study medication or procedure, the following points should be taken into consideration:

- close timely relationship between administration of study medication or procedure and occurrence of AE
- information about the side effect profile of the study medication or procedure from non-clinical and clinical studies
- mechanism of action of study medication or procedure potentially explaining the occurrence of the AE
- other factors with causal potential
- impact of concurrent therapeutic or diagnostic measures
- personal physical or psychological stress factors on the participant’s side

Serious adverse event (SAE)

A SAE is an AE with at least one of the following characteristics:

- results in death
- is life-threatening
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- is a congenital anomaly or birth defect
- is an important medical event

NOTE: The following hospitalizations are not considered SAEs:

- a visit to the emergency room or other hospital department lasting less than 24 hours that does not result in admission (unless considered an “important medical event” or a life-threatening event)
- elective surgery planned before signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)
- medical/surgical admission for purpose other than remedying ill health state that was planned before study entry. Appropriate documentation is required in these cases.
- admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative).

Serious adverse reaction (SAR)

A SAR is an AE which is at least possibly related to the study medication or procedure and fulfils at least one of the criteria of seriousness listed above.

A suspected expected serious adverse reaction (SESAR) during a clinical trial is an AE which is at least possibly related to the study medication or procedure, fulfills at least one of the criteria of seriousness listed above and is listed as potential side effect of the study medication or procedure in the Summary of Product Characteristics or the Investigators’ Brochure.

A suspected unexpected serious adverse reaction (SUSAR) during a clinical trial is an AE which is at least possibly related to the study medication, fulfils at least one of the criteria of seriousness listed above and is not listed as potential side effect of the study medication or procedure in the Summary of Product Characteristics.

New events

A new fact is defined as any new safety data, which could lead to a reassessment of the risk/benefit balance of the study or of the investigational product or that could be sufficient to consider changes in the drug administration or in the pursuit of the study

9.3 Expected adverse reactions

Reference safety document: The current Investigator brochure will be considered as the reference safety document.

Expected Adverse Reaction related to injection of allogeneic bone marrow mesenchymal cell (undetermined frequency, not severe except for immunological reaction)

- transient lumbar pain
- elevation of the body temperature (>37,5°C)

- shivering
- pruritus
- immunological reaction (reactivity of the skin and mucosa – see below) (very rare < 0.5%, severe)

Expected Adverse Reaction related to graft rejection of allogeneic bone marrow mesenchymal cell (very rare < 0.5% but severe)

- skin reactions : rash, pruritus, desquamation, itches
- digestive tract reactions : diarrhoea, cramps, decreased appetite, weight decrease, nausea
- hepatic reactions : jaundice, abnormal liver enzymes, abdominal pain
- pulmonary reactions : lung infection, interstitial lung disease
- another organ may be affected : eyes (dry eyes syndrome, blurred vision, eye pain, runny pain, sensitivity to light), mucosa (dry mouth, difficultyto eating, dental caries, gum disease; genitals disease…), muscles…

Expected Adverse Reaction related to local intra-discal injection

- headache and nausea (undetermined frequency, not severe)

The following adverse reactions are severe but rare

- local infection (discitis),
- reherniation in operated segments,
- exacerbation of pain, back pain, ischialgia, sciatica, pain in extremity,
- facet joint syndrome,
- meningitis,
- epidural abscess,
- arachnoiditis,
- intrathecal hematoma,
- retroperitoneal hematoma,
- cauda equina syndrome,
- acute disc herniation,
- paralysis,
- cerebrospinal fluid leak.

Expected Adverse Reaction related to the concomitant medication

Cf. the current Summary of Product Characteristics (SmPC) to the product.

Expected Adverse Reaction related to MRI

Panic attack

Expected Adverse Reaction related to local anesthesia

- Related to local anesthesic or antiseptic :

Cf. the current Summary of Product Characteristics (SmPC) to the product (1% xylocain for local anesthesic, and chlorhexidine for antiseptic)

- Related to the procedure/technique of local anesthesia:

Anesthesia failures and insufficiencies

Hematomas and arterial dissections

Infections

Neurological lesions (traumatic nerve location or intraneural injection or injected solution toxic)

Error on injected product (Ex: antiseptic, physiological saline)

9.4 Unblinding procedure

This trial is a double blind clinical study for both patient and investigator in charge of the patient evaluations. The knowledge by investigator of the treatment administrated in the study may be necessary but should be exceptional and justified in case of SAEs or specific situations by the adaptation of patient care and medical care depending on the treatment received

SAEs and / or situations that could justify unblinding request on medication administrated as part of the protocol are defined below:

a. Infections requiring hospitalization and administration of IV antibiotics:

b. Unexplained or possibly toxic death.

c. Request by health professional (emergency room, intensive care ...) not involved in the study, the patient himself or his entourage in an immediate emergency.

Concerned patient by unblinding procedure will not be withdrawn of the study

9.5 Investigator responsibilities

AEs

Complete and appropriate data on all AEs (only clinically significant event or value) experienced during the clinical trial should be recorded on the AE form of the CRF on an ongoing basis for the duration of the study. Each AE report shall include a description of the event, an assessment of its seriousness according to the criteria listed above, its duration, intensity, relationship to the study medication, other causality factors (if any), any concomitant medication dispensed, actions taken with the study drug or other therapeutic interventions and outcome at the end of the observation period.

The adverse events will be encoded and graded according to CTCAE Criteria Grade 1-5 (Common Terminology Criteria for Adverse Events). A copy of the CTCAE Version 4.0 can

be downloaded from the CTEP homepage (http://ctep.info.nih.gov). If there is no CTCAE grading possible, the intensity of the adverse event has to be classified in categories:

- Mild (Grade 1) – transient or minimal symptoms; no change in activity or need of medication
- Moderate (Grade 2) – symptomatic; moderate change in activity; no reduction in social activities
- Severe (Grade 3) – incapacitating; bed rest required; loss of work; reduction in social activities
- Life threatening (Grade 4) – significant clinical intervention or hospitalization required
- Fatal (Grade 5)

For each AE, a separate AE form will be filled in.

Follow up of AEs: AEs will be followed until their resolution or stabilisation. However, the observation period will be cut off after the last patient has finished his final visit (LPLV). During monitoring visits, the study monitor will assess accuracy and completeness of all AE forms generated since the last visit by 100% source data verification. Cases of AEs reported earlier for which the outcome is still pending will be checked for new data and developments. Furthermore, queries on AEs reported earlier will be solved with the Investigator. AEs will be reported in the final study report.

The sponsor must be informed immediately of the occurrence of any AE grade 3-4-5 by the associated centre.

SAEs

The Investigator must also record all SAEs in the CRF. For each SAE an additional paper based SAE-Form has to be filled in.

The investigator shall report all SAE immediately involving study participants, including suspected predefined gastro-intestinal, cerebro-vascular and/or hepatic events for assessment and adjudication in a blinded manner by the vigilance department of the sponsor, except for those that the protocol identifies as not requiring immediate reporting such as:

- Specific hospitalisation (Definition of SAEs)
- Serious adverse events occurring after given informed consents, but treatment administration (Visit 1).

These identified SAE will be documented on the CRF/ source data as AE.

The Pharmacovigilance Clinical Trial Department of the sponsor must be informed immediately (i.e. within 24 hours) of the occurrence of any SAE by fax or e-mail (contact details see below).

The Pharmacovigilance Department will confirm receipt of the initial SAE report by return fax or e-mail and provide a SAE case identification number. As on the CRFs, data should be transmitted in pseudonymised form, yet allow identification of the subject by the Investigator.

Causality assessment: The Investigator is required to include any investigations as may be indicated to elucidate the nature of or the causality of the SAE. This may include additional lab tests, histo-pathological examinations, and consultations with other healthcare professional or if the patient dies, any post mortem findings.

Follow up: After the initial report, the Investigator is required to follow each patient and to provide further information on the patient's condition to the sponsor. The Investigator will ensure that the follow-up includes any further investigations as may be indicated to elucidate the nature of the causality of the SAE. This may include additional lab tests, histo-pathological examinations, and consultations with other healthcare professional or if the patient dies, any post mortem findings.

Pharmacovigilance Clinical Trial Department CHU Montpellier

Perrine ROBIN

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9.6 Sponsor responsibilities

Serious Adverse Event and new event

The relation between the event and the study drugs should be evaluated by the sponsor. The sponsor will evaluate whether the events are expected or unexpected.

The sponsor will report any SUSAR and new events according to the legislation and good clinical practice. Any Serious Adverse Reaction related to injection of allogeneic bone marrow mesenchymal cell, to graft rejection of allogeneic bone marrow mesenchymal cell, to local intra-discal injection and those related to bone marrow donation will be declared to the corresponding national competent authority in the same way that SUSARs

Regulatory declaration is made within a maximum of:

- without delay for serious adverse unexpected fatal or life-threatening. In this case, additional relevant information should be sought and passed within a further period of 8 days.

- 15 calendar days for all other serious unintended effects. The same additional relevant information should be sought and transmitted in a further period of 8 days.

Assessment of Adverse Events

The assessment of the relationship of an AE to the administration of Drug Product is a clinical decision based on all available information at the time of, and after the occurrence of the event. The factors to be considered when evaluating the relationship of an AE to the Drug Product include:

- Temporal sequence from drug administration: the event must occur after the Drug Product is given. The length of time from exposure to medication to event should be evaluated in the clinical context of the event.

- Recovery on discontinuation (dechallenge), recurrence on reintroduction (rechallenge).
- Underlying, concomitant, intercurrent diseases: each report should be evaluated in the context of the natural history and course of the disease being treated and any other diseases the patient may have had prior to, or developed during the course of the study.
- Concomitant medication or treatment: the other drugs the patient is taking or the treatment the patient is receiving at the time of the event should be examined to determine whether any of them may be recognized to cause the event in question.
- Known response pattern for this class of drug.
- Exposure to physical and/or mental stress: the exposure to stress may induce adverse changes in the patient and may provide a logical explanation for the event.
- The pharmacology and pharmacokinetics of the Drug Product: absorption, distribution, metabolism and excretion of the Drug Product or other medications the patient is receiving, coupled with the pharmacodynamic responses, should be considered when evaluating an event.

From a statistical and regulatory point of view, an AE will be categorized as “reasonable” or “not reasonable” related to Drug Product.

Expedited reporting

Other safety issues also qualify for expedited reporting where they might materially alter the current benefit-risk assessment of the investigational medicinal product or that would be sufficient to consider changes in the investigational medicinal products administration or in the overall conduct of the trial, for instance:

a) an increase in the rate of occurrence or a qualitative change of an expected serious adverse reaction (SESAR), which is judged to be clinically important,

b) post-study SUSARs that occur after the patient has completed a clinical trial and are reported by the investigator to the sponsor, up to 1 year follow up

c) new events related to the conduct of the trial or the development of the investigational medicinal products and likely to affect the safety of the subjects, such as:

d) a SAE which could be associated with the trial procedures and which could modify the conduct of the trial,

e) a major safety finding from a newly completed animal study (such as carcinogenicity)

f) recommendations of the IEDSMB, if any, where relevant for the safety of the subjects

Development Safety Update Report (DSUR)

In addition to the expedited reporting, the sponsor shall submit, once a year (on the anniversary of the first approval date of the clinical trial by regulatory authorities), throughout the clinical trial or on request, a safety report to the competent authorities and the Ethics Committee of the concerned Member States taking into account all new available safety information received during the reporting period.

The aim of the DSUR is to describe concisely all new safety information relevant for one or several clinical trial(s) and to assess the safety conditions of subjects included in the concerned trial(s).

It should be the same for the competent authorities concerned and the Ethics Committee concerned.

The Sponsor is responsible for the ongoing safety evaluation of the Investigational Product. The Sponsor should promptly notify all concerned investigators and the regulatory authorities of findings that could affect adversely the safety of subjects, impact the conduct of the trial, or alter the CA approval/ EC's favourable opinion to continue the trial.

9.7 Data and Safety Monitoring Board

This clinical trial will be followed by an Independent Ethic Data Safety Monitoring Board (IEDSMB), which will review the safety data and provide recommendations to the Sponsor regarding the safety of subjects, the conduct of the study and potential premature termination.

An IDSMB is an independant consultative board asked to express an opinion to the sponsor of the study on the benefit/risk ratio and the management of the clinical trial.

An IEDSMB will be set up composed of the following members, at least:

- A clinician specialist in immuno-rheumatology to evaluate the clinical aspects of safety and efficacy of the study
- A pharmacologist with skills on the mode of action and safety of the concerned product
- A methodologist or statistician independent

In particular, The IEDSMB will :

- provide independent, competent, and timely review of the data quality and the safety of the clinical trial.
- have appropriate independence from political, social, institutional, professional, and market influences, as well from the sponsor.
- have no direct relation with the ethicists of the PAB. However, all amendments and revisions that received a positive feedback from the PAB will be submitted to theIEDSMB, whether or not related to recommendations of the IEDSMB. Site-specific amendments may require special treatment.
- will meet at regularly defined intervals via teleconference to review and evaluate the quality of data collected during the clinical trial and assess reports on cumulated serious adverse events, as per the IEDSMB chart developed for the clinical trial and provide advices to the PAB.

The selection of members must be collegiale (investigator-coordinator, sponsor) and, for the IEDSMB meetings, must be made by quorum is reached when a majority of members are present (half members +1 and at least 3 members out of 4).

The members must appoint a chairman, which is the main interlocutor of the sponsor. He is in charge with the drafting of reports and the opinions delivered.

The members are nominated and authorized by the sponsor for the duration of the study. They agree on their participation as volunteers as on the respect for the confidentiality of the data.

The IEDSMB receives successive versions of the protocol, DSUR, and can be requested at any time by the sponsor if a SUSAR or SAEs involves particular problems for analysis, if data may modify the benefit/risk ratio appear under study.The IEDSMB analyses data that are transmitted, may request additional information. It makes recommendations about the future of the study (continuation, amendment, stop). The report or opinion of the IEDSMB is transmitted as soon as possible to the sponsor. It will be transmitted by the sponsor to the coordinating investigator, Ethics Committee, and the Competent Authority, as part of annual safety report of the trial. Upon receipt of the opinion of the IEDSMB, the sponsor deliberates and makes its decision. It is usual to comply with the opinion of the IEDSMB, but there is no obligation because this opinion is advisory. Nevertheless, a strong difference of opinion implies that the reasons are justified in writing for the IEDSMB, the coordinating investigator, the Competent Authority and Ethics Committee.

Composition and meeting modalities are defined in the IDSMB Charter.

The IEDSMB will be set up and will have a first meeting before the start of the study.

10 Data handling and record keeping

It is the responsibility of the Sponsor to ensure that the clinical trial is conducted according to all stipulations of the protocol and in accordance with ICH-GCP, the Declaration of Helsinki and local regulatory requirements.

The Sponsor or his designee must ensure that data are recorded in the eCRF correctly and completely by authorized personnel. The investigator has to confirm the integrity of the data transferred to the eCRF by signature.

The investigator is responsible for the completion and maintenance of the confidential patient identification code which provides the unique link between named patient source records and pseudonymized eCRF data. The investigator must arrange for the retention of this patient identification log in the ISF..

The principal investigator of the site must provide a staff signature list to determine the responsibilities of each person of the trial personnel. The staff signature list has to be kept in the ISF and TMF.

10.1 Investigator site file (ISF)

The investigator is responsible for maintaining all records which enable the conduct of the clinical trial at the site to be fully documented, in compliance with ICH GCP filing standard. Timeliness and completeness of the documentation is regularly checked by the clinical monitor.

10.2 Trial Master file (TMF)/Country Master file

The sponor and each country correspondent for the clinical trial management (regular submissions/visits) will maintain, respectively the TMF and the country master file with relevant document of the clinical trial according to ICH/GCP filling standard.

10.3 Obligation to archive (Principal investigators and sponsor)

All completed study related documents (e.g. eCRF, Informed consent forms, drug accountability logs, staff signature lists, Subject identification log, study initiation visit and monitoring visits reports) must be archived by each investigator for its centre and by the sponsor for 30 years.

10.4 Adherence to the clinical trial protocol

Protocol violations are any deviations from the procedures outlined in this document, for example, missed evaluations, incorrect timing of evaluations, non-compliance with GCP and intake of prohibited medications. It is the investigator's responsibility to make all reasonable efforts to avoid protocol violations in order to avoid possible exclusion of the patient from the study and/or analyses.

An effort should be made to ask the sponsor for permission to deviate from the protocol if really necessary. Only if the safety of a patient is in immediate danger, the investigator may deviate from the protocol on his own responsibility.

All protocol violations will be reported immediately to the Sponsor or his delegate and any action required, for example, discontinuation of the patient will be discussed. Evaluability of the patient(s) concerned will be performed by the IEDSMB prior to the statistical analysis.

Any deviations from the protocol that has not been approved by the Sponsor or his delegate, the Competent Authority and the concerned EC could result in a discontinuation from the study of the site involved.

11 Data collection and data management

Data collection will be performed with electronic Case Report Forms (eCRF). The data management will follow a Remote Data Entry approach. The eCRF will be implemented in a modern Clinical Data Management System (CDMS) with Electronical Data Capture functionality (EDC) available at IZKS Mainz. For the system used, MACRO, Elsevier, a system validation has been performed and GCP-compliant data management at IZKS Mainz has been certified by ECRIN in July 2016. Implementation of the eCRF will be performed by data managers from IZKS Mainz in cooperation with biostatisticians from CHU of Montpellier. The system complies with the relevant international regulations and standards and provides the capability to perform the major data management activities within a consistent, auditable and integrated electronical environment (query management, data entry, data validation). Range, validity and consistency checks will be implanted in the eCRF for application during data entry. There will be a comprehensive validation of the eCRF before release to the investigators. All data entry, modification or deletion will be recorded automatically in an audit trail indicating the original value, the new value, the reason for change, who made the change and when the change was made. A digital signature is implemented and included in the audit trail. The internet connection is secured by adequate technology. The computerised system is able to generate accurate and complete copies of records in both human and electronic form for inspection, review and copying by regulatory authorities and ethics committees.

IZKS Mainz will supply the necessary documentation and training to the investigator, study site staff and study coordinator representatives, and will support the use of the eCRF

through a help-. During the trial all findings will be documented on eCRFs or other study specific paper forms by the responsible investigator or designated representatives. The investigator will maintain a list of these representatives. Paper forms will be signed by either of them. The data has to be complete, clear, accurate, legible, and plausible. Missing examinations or dates have to be marked along with a justification/explanation. Corrections on study paper forms are to be made according to GCP guidelines, i.e. the version that has to be corrected will be crossed in a way that it is still readable, the corrected version will be written above or beside the final version and the correction (or any remark) will be marked with data, initials, and a justification by the investigator or an authorized person. The query management is performed electronically under the supervision of the monitor. Data corrections in the eCRF, if necessary, have to be performed by the Investigator or designated representatives. Only these persons are allowed to the system and their identity during use will be registered. Data on patients collected on eCRFs in the course of the trial will be documented in a pseudonymous fashion. For monitoring and auditing purposes, and to the greatest extent possible, all information must be traceable back to the source documents, which are generally maintained in the patient's file. The source documents should cover demographic and medical information, including laboratory data, medication, physical examination, etc.

12 Quality control and quality assurance

12.1. Site Training and Monitoring Procedures

The Monitor must present the protocol and all procedures related to the study during an initiation visit performed before the first patient is included. A case report form completion guidelines will be provided to the Investigator by **IZKS Mainz**.

12.2. Direct access to source data/original documents

The Monitor will be allowed to have access to all source documents needed to verify the entries on the eCRF and other protocol-related documents.

12.3. Accuracy and Reliability of Data

To ensure accurate, complete, and reliable data, the sponsor or its representatives will do the following:

a. Provide instructional material to the study sites, as appropriate.
b. Provide a start-up training session to instruct the investigator(s) and study coordinator(s). This session will give instruction on the protocol, the prompt and full completion of the clinical report forms, study procedures, and the transmission of data in a timely manner to the clinical database for statistical analyses.
c. Make periodic visits to the study site.
d. Be available for consultation and stay in contact with the study site personnel by mail, telephone, and/or fax.
e. Review and evaluate case report form data and use.

f. Conduct quality review of database.

In addition, the sponsor or its representatives may periodically check a sample of the subject data recorded against source documents at the study site. The study may be audited by the sponsor and/or regulatory agencies at any time. Investigators will be given notice before an audit occurs.

To ensure the safety of participants in the study and to ensure accurate, complete, and reliable data, the investigator will keep records of laboratory tests, clinical notes, and subject medical records in the subject files as original source documents for the study. Investigator files will identify whether any clinical report form entries are source data. If requested, the investigator will provide the sponsor, applicable regulatory agencies, and/or applicable ethical review boards with direct access to original source documents.

The investigator has the responsibility of explaining the correct use of the investigational agent(s) to the subject and site personnel, ensuring that instructions are followed properly.

13 Monitoring, audits and inspections

13.1 Monitoring

Monitoring will be performed every 2 to 3 months during the whole study at investigating sites and pharmacies according to the sponsor specific SOP (monitoring plan). The monitoring will include clinical parameters (VAS, Oswestry, SF-36 questionnaires), MRI, AE.

Routine monitoring visits will be made by the monitors designated by the Sponsor to check compliance with the protocol, the completeness, accuracy and consistency of the data, and adherence to GCP.

The principal investigator must ensure that eCRFs are completed in a timely manner and must allow periodical access to eCRFs, patient records, drug logs and all other study-related documents and materials. The frequency of monitoring visits will be determined by factors such as study design and the site enrolment requirements but visits will normally occur at least once every 2-3 months.

The investigator will agree to provide the monitor direct access to the subjects' source data, which may exist in the form of hospital records, patient files and notes, and laboratory assessment reports and results.

13.2 Audit and Inspection

The purpose of an audit is to confirm that the study is conducted as per protocol, ICH-GCP and applicable regulatory requirements, that the well-being and the rights of the subjects enrolled have been protected, and that the data relevant for the evaluation of the investigational Medical product have been recorded, processed, and reported in compliance with the planned arrangements. The investigators will permit a direct access to all study documents, drug accountability records, medical records and source data.

14 Informed Consent, Ethical Review, and Regulatory Considerations

14.1 Informed consent

Informed consent must be obtained from each subject ~~or legal representative~~, where the person is not capable of giving consent, before the performance of any study-related activity.

The investigator or an authorized associate must explain the nature of the study and the treatment in such a manner that the subject is aware of his/her rights and responsibilities, as well as potential benefits and risks. The investigator is also responsible for answering any questions the subject may have throughout the study and for sharing any new information, in a timely manner, that may be relevant to the subject's willingness to continue his/her participation in the study. The Informed Consent Form (ICF) and Patient Information Leaflet (PIL) also include indications about the insurance coverage and the resulting regulations for the delimitation of damages. The subject will be informed that he/she should notify the investigator of any other medical measures during the study period and that he/she cannot simultaneously take part in another study.

Subjects must also be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice to their current or future care. Documentation of the discussion and the date of informed consent must be recorded in the subject's medical record.

Concerning the study data, by signing the ICF, the patient will accept that the study data may be examined by the Sponsor, the CAs, ECs, a mandated auditor and/or the study monitor in compliance with the statement of confidentiality.

Subjects or legal representative must sign and date the ICF after the nature of the study has been fully explained. A copy of the completed ICF as well as the PIL must be provided to the subject. Before its use, the PIL and ICF must meet local regulations and be approved by the EC

14.2 Ethical review/National competent authority approval

Requirements for ethical review and national competent authorities for each country as set forth in Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice (GCP) in the conduct of clinical trials on medicinal products for human use or other relevant local regulations for institutional review will be followed. The Protocol, ICF/PIL, Investigator's Brochure and other required documents must be approved by the EC and NCA before enrolment of subjects in the study. The Sponsor must confirm that the EC is in compliance with the general standards for the composition, operation, and responsibility of an EC as set forth in ICH Guidelines for GCP. The letter of approval from the EC, the NCA, as well as a list of documents reviewed, will be filed in the Investigator Site File (ISF) and a copy will be filed in the trial master file (TMF) held by the Sponsor.

Any member of the EC who is directly affiliated with this study as an investigator or as site personnel must abstain from the EC vote on the approval of the protocol. The Sponsor, in collaboration with the investigator, will be responsible for reporting to the EC and to the NCA all changes in research activity, including protocol amendments, updates of Investigator's Brochures, annual safety reports, all unanticipated problems involving risks to

human subjects, and study termination. The investigator will also be responsible for submitting progress reports to the EC at regular intervals appropriate to the degree of subject risk involved, but no less than once per year. Copies of all EC and NCA notifications and approvals will be forwarded to the Sponsor.

The site will also apply to their Local Authority and Ethics Committee (EC) as appropriate for approval to participate in the study.

14.3 Regulatory Considerations

14.3.1 Responsibilities of the sponsor and investigators

Prior to initiating the clinical trial, the sponsor or his delegate defines, establishes and allocates all trial-related duties and functions. The sponsor or his delegate ensures that all investigators are provided with instructions and a uniform set of standards for the assessment of clinical and laboratory findings, and on completing the eCRFs.

The different partners have to declare no conflicts of interest.

14.3.2 Responsibilities of the Investigators

The investigators agree with the requirements of the signed protocol. The investigator's responsibilities shall include but not be limited to:

- Knowledge of the properties of the IMP and familiarity with the appropriate use of the investigational medicinal product as described in the in the Investigator's Brochure
- Detailed knowledge of the clinical trial protocol
- To have sufficient time, an adequate number of qualified staff to conduct the trial properly and safely
- To ensure that adequate medical care is provided to a subject for adverse event related to the trial
- To ensure accuracy, completeness, and timeliness of the collected data, documents and reports
- Data reported on the eCRF should be consistent with source data
- Upon request of the monitor, auditor or regulatory authority, direct access to all trial-related records should be permitted
- To ensure that patient's anonymity is maintained
- To declare financial interests in the clinical trial and IMP if applicable

The investigators are responsible for the conduct of the clinical trial at the respective site. In signing this protocol, the Investigator accepts to carry out all procedures related to this study according to the laws and guidelines of the EU regarding the conduct of clinical research and any local requirements of the individual EU country. Investigators must allow access to all documents pertinent to the study. In particular, the Investigator must comply with current international conference on harmonization (ICH) tripartite guidelines for good clinical practice (GCP) and current EU Directive on clinical trials (Directive 2001/20/EC of the European parliament and of the council of 4 April 2001). The study may be subject to inspection by Regulatory Authorities or Sponsor's audit and will be monitored by accredited personnel. The Protocol must be read thoroughly and the instructions herein must be

followed exactly. Any deviations should be agreed between the Sponsor and the Investigator before the occurrence of the deviation, with appropriate written protocol deviations made to reflect the changes agreed upon. Where the deviation occurs for the well-being of the patient, the monitor must be informed and a course of action agreed. If the Investigator moves, withdraws from the study or retires, the responsibility for conducting the study and maintaining the records may be transferred to another Investigator at the same centre who will accept responsibility for taking over the study. Notice of transfer must be made to, and agreed by the Sponsor.

14.3.3 Patient confidentiality

Recording, transmission and storage of subjects' trial-relevant data will be performed according to local secrecy obligations, as well as national and European requirements (EU Directive 96/46 on data protection).

The principal investigator must ensure that the patient's anonymity is maintained. On the eCRFs or other documents submitted to the Sponsor, subjects should not be identified by their names, but by their assigned identification number. If patient names are included on copies of documents submitted to the Sponsor, the names must be obliterated and replaced with the assigned study patient numbers.

Participants are separately informed about data security in the patient information leaflets /informed consent form.

The principal investigator should keep a separate log of patient identification numbers, names, addresses, telephone numbers and hospital numbers (if applicable). Documents not for submission to the Sponsor, such as signed informed consent forms, should be maintained in strict confidence by the principal investigator in the ISF.

A screening failure log will be maintained for subjects who have consented to participate in the study but who, for whatever reason, are not eligible, withdrawn or decide to withdraw prior to taking part. This log will contain the following information:

- Patient study number.
- Reason for study withdrawal (when available).

eCRF pages will not be completed for these subjects.

The investigator shall permit authorised representatives of the Sponsor, regulatory authorities and IECs to review that portion of the patient's medical record that is directly related to the study. As part of the required content of informed consent, the patient must be informed that his/her records will be reviewed in this manner.

14.3.4 Good Clinical Practice

This study will be conducted in accordance with the protocol and ethical principles stated in the Declaration of Helsinki or the applicable guidelines on GCP, and all applicable local laws, rules, and regulations.

All data recorded in the case report form (eCRF) for subjects participating in this study will be transcribed from source documents.

In parallel to the submission to the EC, the Sponsor has to obtain an authorisation from the appropriate competent authority (CA) to conduct the clinical study. Subjects must not be entered into the study until the relevant EC has issued its opinion and the CA has given authorisation to conduct the study.

All substantial amendments must be submitted to the EC and/or to the CA for approval.

14.3.5 Amendments to the clinical trial protocol

The investigator should not implement any deviation from, or changes of the protocol without agreement by the sponsor or his delegate and prior review and documented approval of an amendment by the competent authority and the concerned ethics committee, except where necessary to eliminate an immediate hazard to trial participants, or when the change involves only administrative aspects, per European law (Directive 2001/20).

14.3.6 Declaration of End of trial

The end of the trial will be notified to concerned ethics committee and competent authority within 90 days, as required by European and local legislations.

14.3.7 Final Report Signature

A clinical study report, written in accordance with ICH Guideline E3, will be submitted in accordance with local regulations and requirements set forth in Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of GCP in the conduct of clinical trials on medicinal products for human use.

14.3.8 Financing and Insurance

The clinical trial is financed by the H2020 Programme of the European Union, under grant agreement number **732163.** No funds from third parties are involved.

The Sponsor or his delegate will procure insurance by Lloyd's France SAS for this clinical trial to cover trial related injuries of the participants according to local regulatory requirements.

Clinical trial participants will be provided on request with the conditions of insurance provided by Lloyd's France SAS along with the patient information and consent form.

14.3.9 Investigator Information

The contact information and qualifications of the principal investigator and sub-investigators and name and address of the research facilities are included in the ISF.

15 Clinical trial registry

The clinical trial will be registered in an appropriate public clinical trial registry before the first participant is enrolled. The registry should meet the ICMJE criteria (e.g. DRKS, ClinicalTrials.gov).

16 Publication policy

Upon study completion and finalization of the study report the results of this trial will be submitted for publication, irrespective of findings.

Investigators may not submit study information for publication without prior consultation of Respinexcom and sponsor.

17 Sponsor, coordinating centre(s) and committees

The CHU of Montpellier will be the Sponsor of the trial, the investigation being conducted in 3 different countries. The management of the study will be conducted at the European level by ECRIN-ERIC with highest quality and according to national and international standards. The Sponsor is responsible for the overall study and it will guarantee that the trial is conducted correctly in compliance with the protocol, ICH-GCP (DIRECTIVE 2001/20/EC)[37], the current version of the Declaration of Helsinki and all applicable regulatory requirements. A quality control system will be established under the responsibility of the sponsor. ECRIN-ERIC will support the Sponsor by providing several coordinated services: regulatory and ethical submissions, monitoring, IMP management support and local pharmacovigilance provided through the national partners of ECRIN for each country, and data management, as a central service, provided through one of the ECRIN-certified data centers compliant with the ECRIN specifications, and with EMA and FDA requirements.

Work-packages: the Coordinator (CHU of Montpellier) and the sponsor team will be assisted by the team of WP2, WP3 and WP4 of the European project to insure that the research performed by the EUROSPINE partners is in accordance with study documentation, relevant regulations and guidelines.

The Scientific Advisory Board (SAB) will provide a scientific follow-up of the clinical trial. A Independent Ethic Data Safety Monitoring Board (IEDSMB) will provide a review the safety data and provide recommendations to the Sponsor regarding the safety of subjects, the conduct of the study and potential premature termination.

[37] DIRECTIVE 2001/20/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 4 April 2001on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use

ANNEXE 1 - Lumbar spine examination (Ombregt L., 2013)

Standing		
Inspection	Deviation	Fig 1.1 (a-b)
	Pain	
	Irregularities	Fig 1.2 (a-b-c-d)
Palpation		Fig 1.3 (a-b-c)
Lumbar movements	Extension Side flexion	Fig 1.4 (a-b-c-d-e)
	Flexion	
Schober test		Fig 1.5
Motor conduction	Standing on tiptoe	Fig. 1.6
Supine		
Mobility of dura mater and nerve roots L4-S2	Straight leg raising test	Fig 1.7a
	Lasègue Test	
	Painful arc	Fig. 1.7b
Motor conduction	Resisted flexion of hip joint	Fig 1.8
	Resisted dorsiflexion of foot	Fig 1.9
	Resisted dorsiflexion of big toe	Fig 1.10
		Fig 1.11
	Resisted eversion of foot	
Sensory conduction	Front of thigh	Fig 1.12
	Front of thigh, inner side of lower leg	
	Big toe	
	Big toe and adjacent toes	
	Outer border of foot and two outer toes	
	Sole of heel	
Knee reflex	Patellar tendon	Fig 1.13

Plantar reflex	Plantar surface of foot	Fig 1.14)
Ankle reflex	Achilles tendon	Fig 1.15
Prone		
Palpation	Lumbar spinous processes	Fig 1.16 et 1.17

STANDING EXAMINATION

INSPECTION

The clinician should observe the patient from the moment he or she enters the consulting room. Next, the patient undresses so that posture can be observed, especially the lower back, pelvis and lower extremities (Fig 1.1).

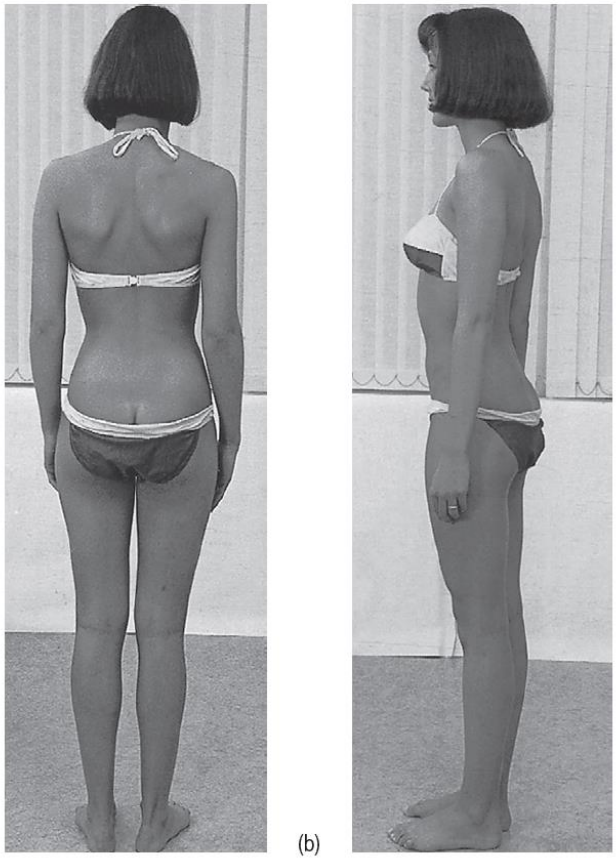


Fig 1.1: The shape of the normal trunk.

Many lumbar spinal disorders present with *asymmetrical posture* (FIg 1.2)

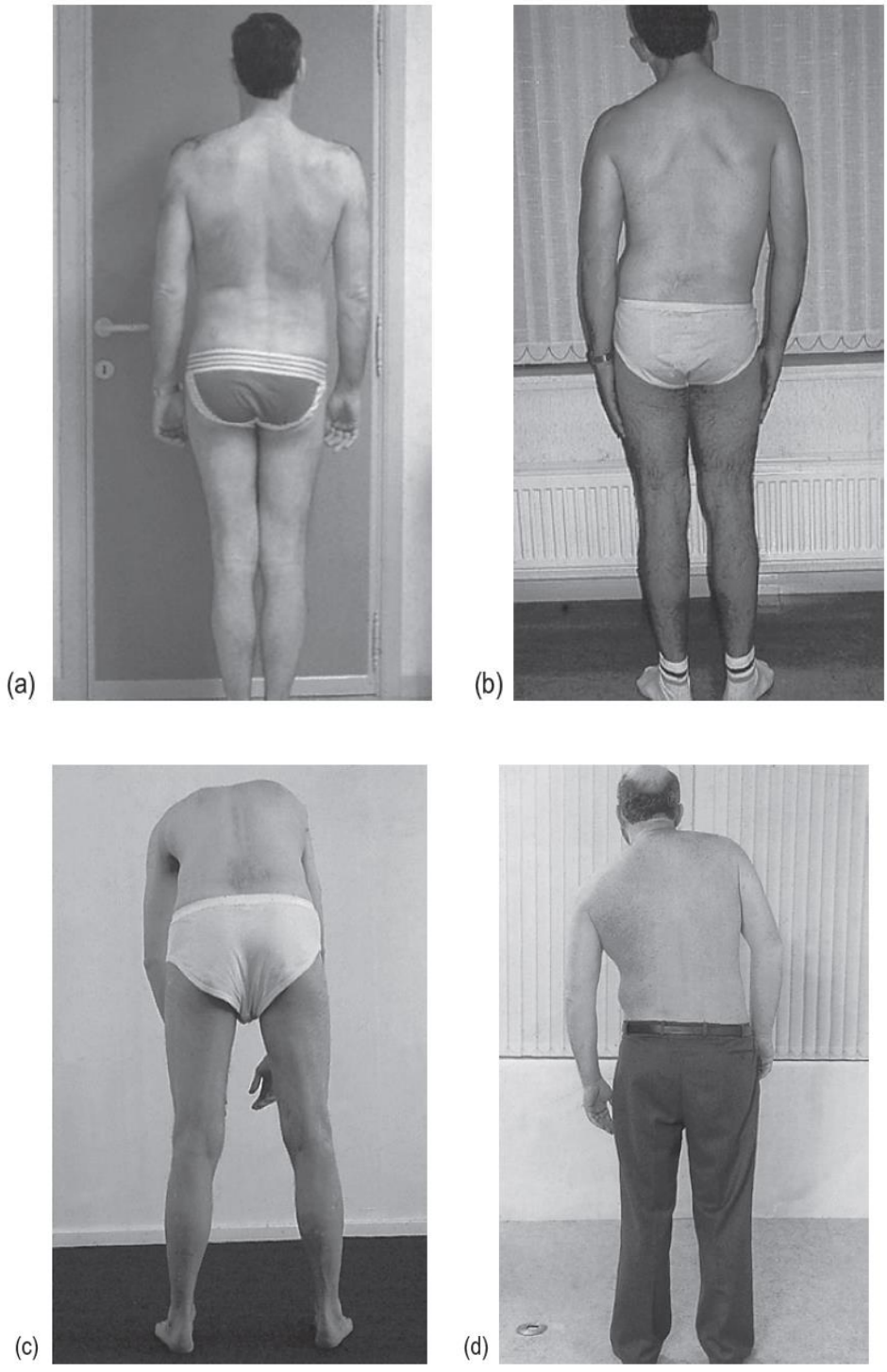


Fig 1.2: Types of scoliosis: (a) static; (b) sciatic; (c) idiopathic; (d) psychogenic.

PALPATION

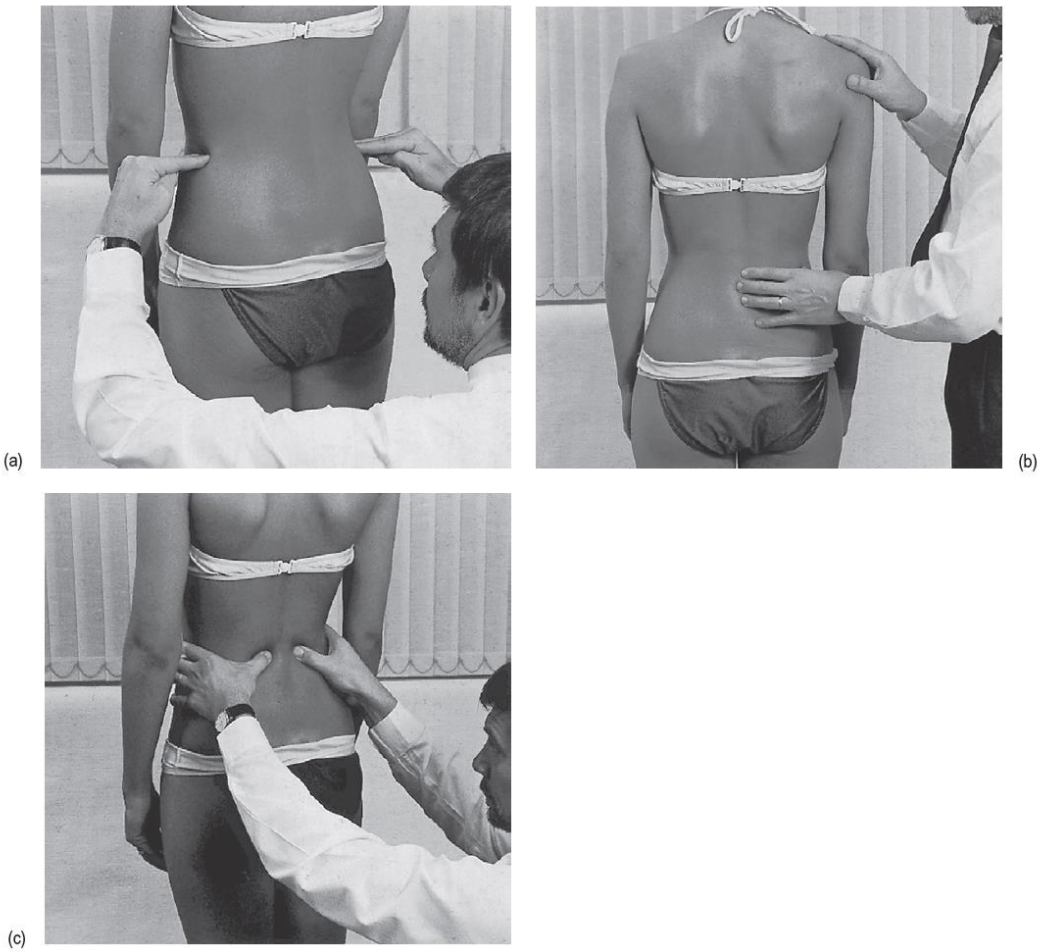


Fig 1.3: Palpation of the iliac crests (a), shelf (b) and muscle spasm (c)

LUMBAR MOVEMENTS

Four active movements are examined while the examiner watches the patient from behind: backward bending, side bending to each side and forward bending completed at full range by neck flexion (Fig. 1.4).

Movements should be performed smoothly and gradually. Any deviation and/or restriction are noted and painfulness ascertained. As a movement is performed, the patient should tell the examiner when pain is felt and where. Momentary pain during the movement (painful arc) should not be missed and is pathognomonic for a disc lesion

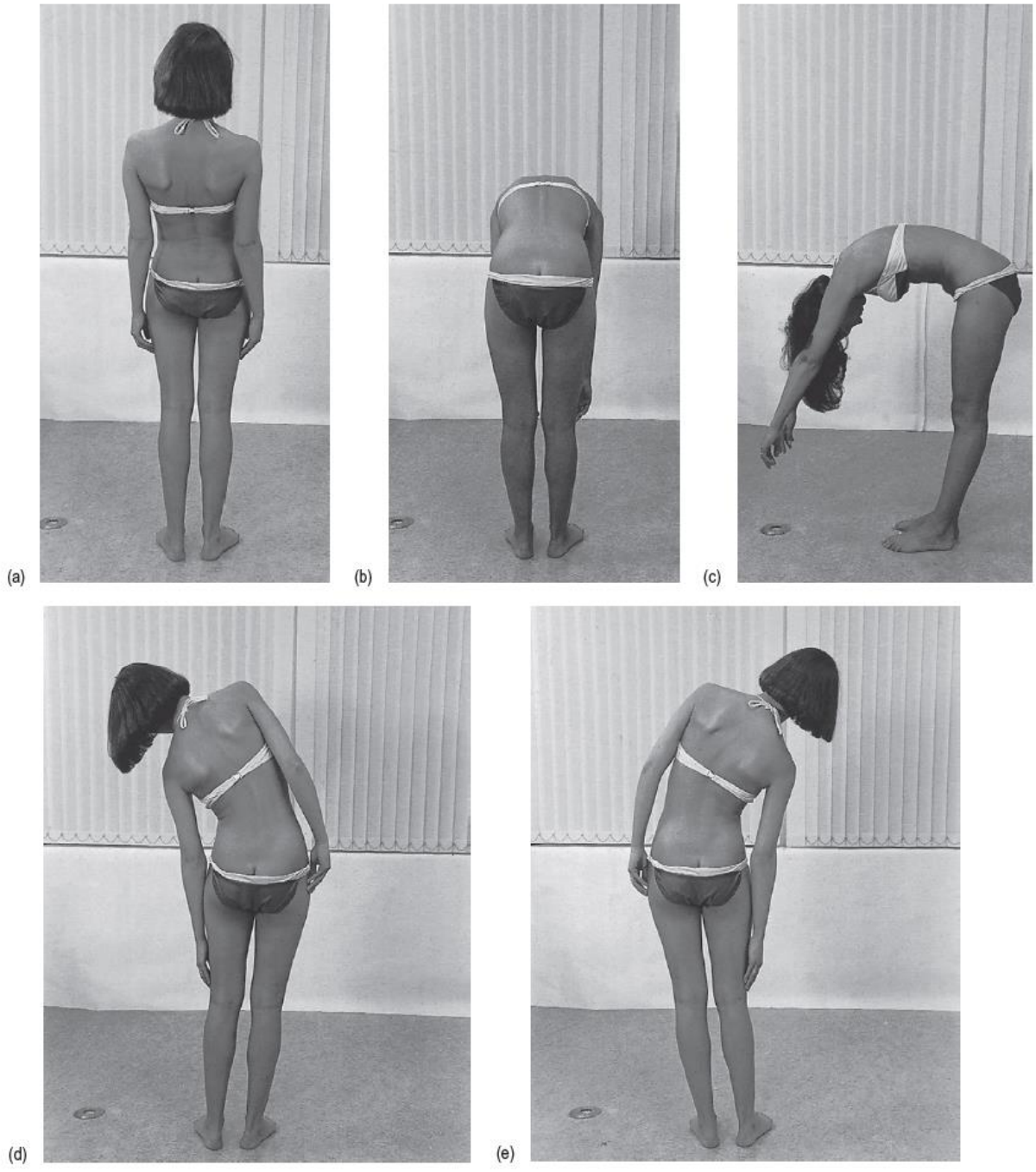


Fig 1.4: Examination standing: (a) backward bending; (b, c) forward bending; (d, e) side bending

SCHOBER TEST

Original Schöber Test: The patient is standing with his back towards the examiner. The examiner determines the location of the lumbosacral junction and marks it by drawing a horizontal line. A second line is marked 10 cm above the first line. The difference between the measurements in erect and flexion positions indicates the outcome of the lumbar flexion. (Rezvani. 2012).

Modified Schöber Index (also called short Schöber test) (Lilius 1989). The patient is standing with his back towards the examiner. The examiner determines the location of the lumbosacral junction by precising the location of the dimples of Venus. The intersection of the top of the dimples of Venus is marked by drawing a horizontal line. This line acts as the landmark. The second line is marked 10 cm above the first and the third is marked 5 cm below the first line. The difference between the measurements in erect and flexion positions indicates the outcome of the lumbar flexion. (Rezvani. 2012)

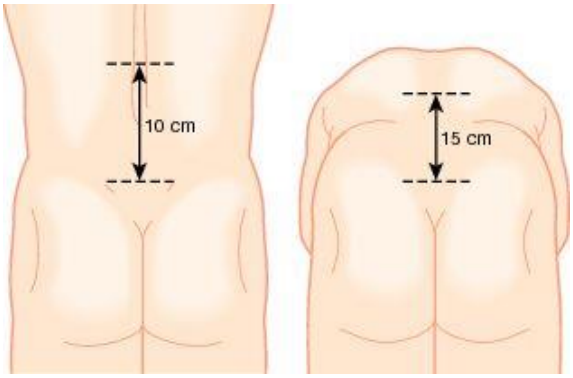


Fig 1.5: Schober test

MOTOR CONDUCTION

The last test in the standing position is standing on tiptoe, which examines the strength of the calf muscles and thus the integrity of the S1/S2 segment. The patient is invited to perform the test, first on the good leg and then on the bad (Fig 1.6).

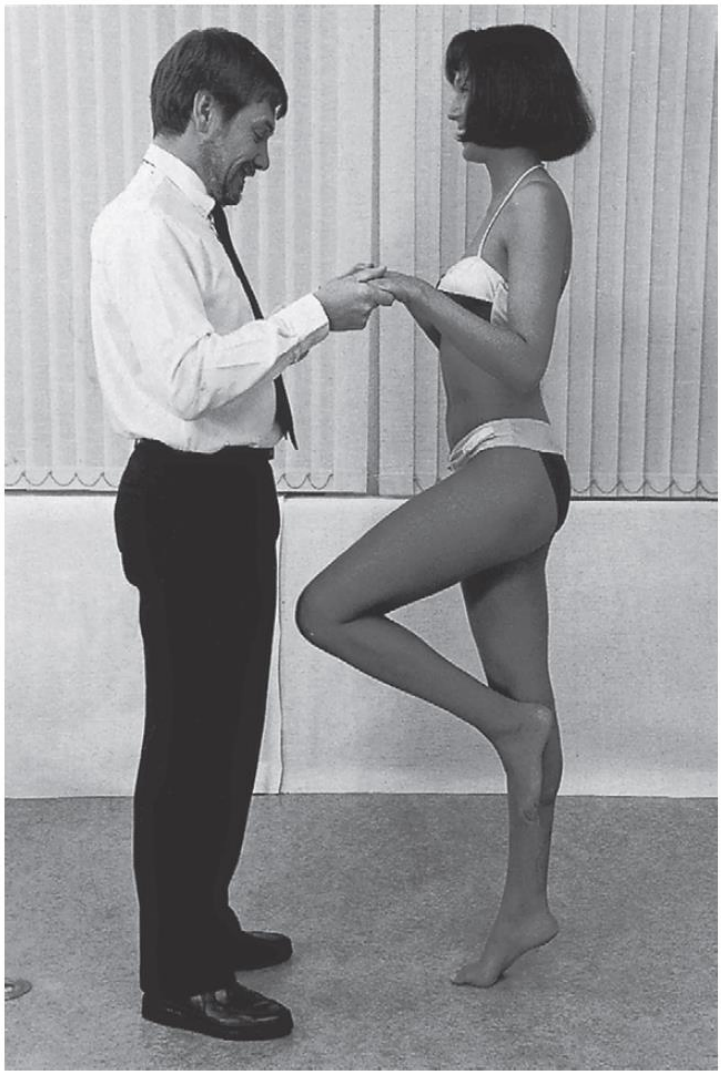


Fig 1.6: Standing on tiptoe

SUPINE EXAMINATION

MOBILITY OD DURA MATER AND NERVE ROOTS L4-S2

Before testing, it should be assumed that there is at least 90° of flexion at the hip joint; otherwise conclusions cannot be drawn. Then the leg is lifted upwards from the anatomical position by supporting the foot at the calcaneus. To prevent the knee from bending, the other hand is placed on its anterior aspect (Fig. 1.7a). The patient should also not be allowed to rotate the pelvis forwards or to abduct and externally rotate the leg at the hip, in order to escape painful stretching.

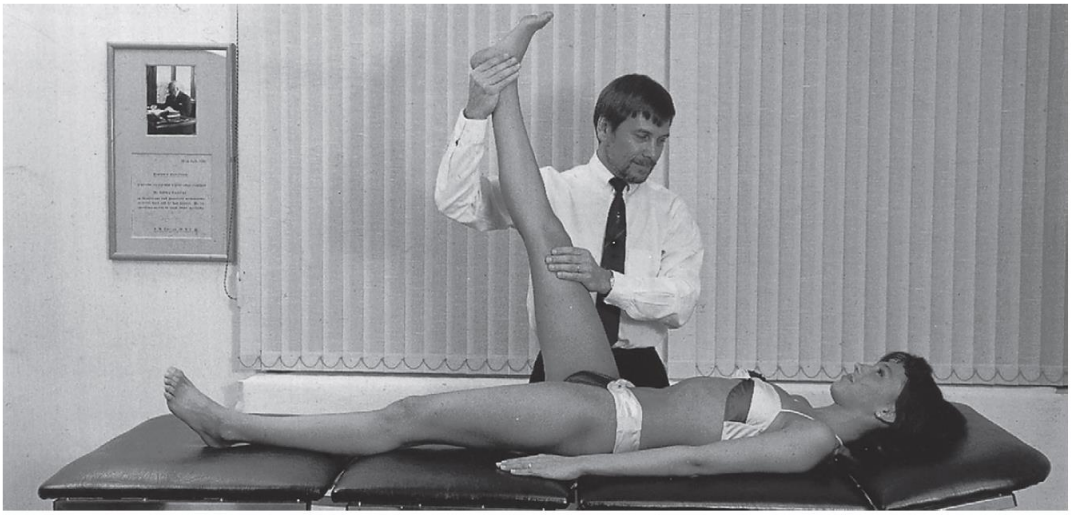


Fig 1.7a: Straight leg raising

PAINFUL ARC : The patient feels a momentary pain on the way up and/or on the way down. A painful arc may be an isolated finding during SLR but is usually seen in combination with pain at full range.

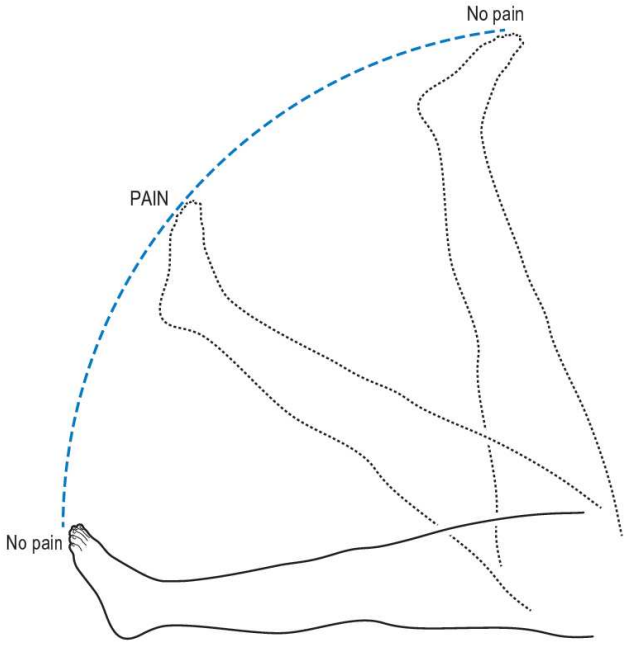


Fig 1.7b: Painful arc on straight leg raising

MOTOR CONDUCTION

The resisted flexion of the hip test is performed with the hip joint flexed to 90° so as to eliminate activity of the rectus femoris as much as possible. Both hands are placed at the distal end of the thigh and the patient attempts to resist the strong force applied by the examiner (Fig. 1.8). At the same time, it is necessary to stabilize the ilium with one knee placed against the patient's ischial tuberosity.

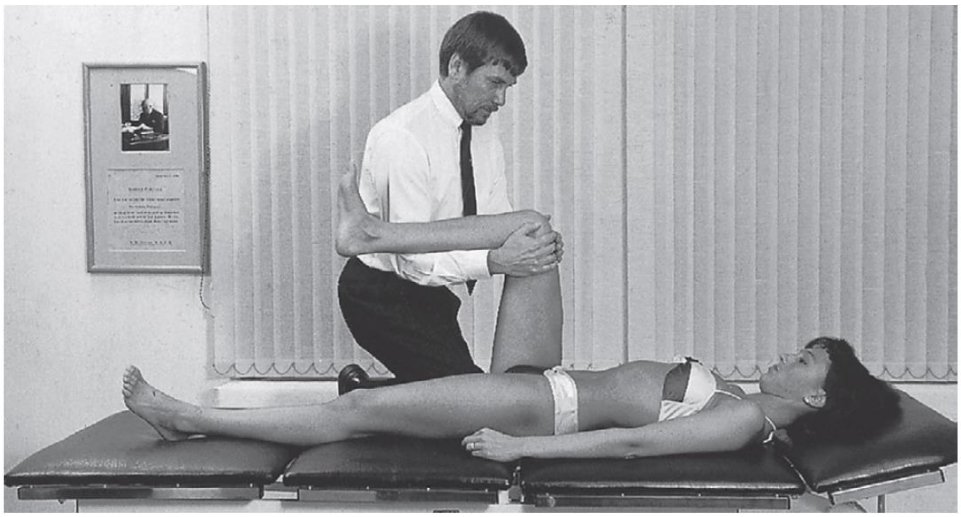


Fig 1.8: Resisted flexion of the hip.

For the resisted dorsiflexion of the foot the patient lies supine with the hips and knees extended. The patient holds the ankle in full dorsiflexion and should resist the full weight of the examiner's body (Fig. 1.9)

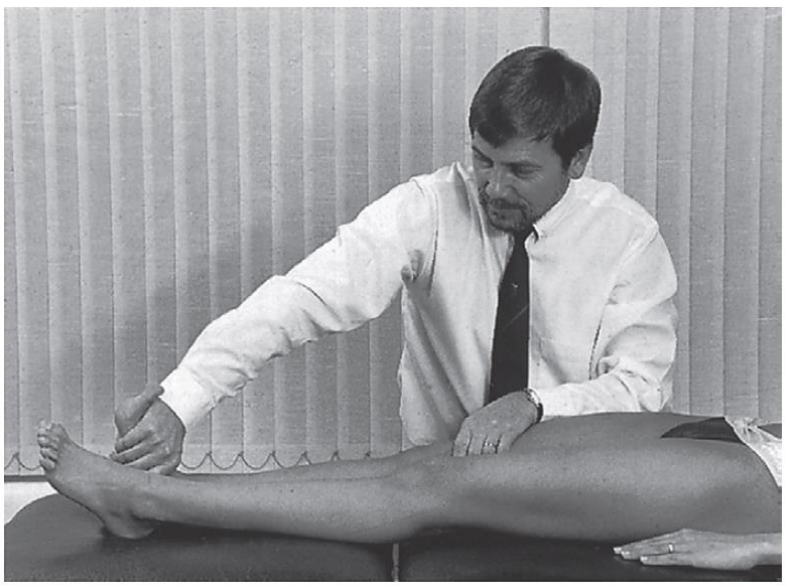


Fig 1.9: Resisted dorsiflexion of the foot.

To perform the resisted dorsiflexion of the big toe, the examiner places the thumb on the nail bed of the great toe and the fingers on the ball of the foot. The patient is asked to resist the examiner's attempt to plantiflex the great toe (Fig.1.10).

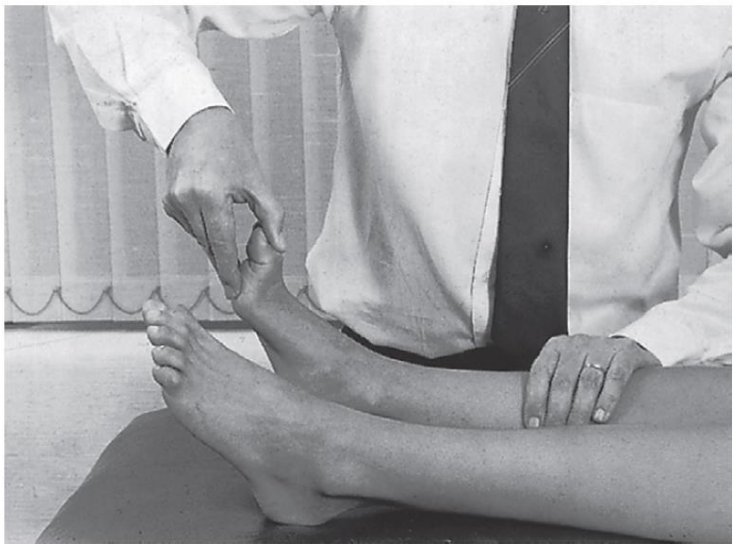


Fig 1.10: Resisted dorsiflexion of the big toe.

To perform resisted eversion of the foot, one hand stabilizes the ankle at the medial side, while the other hand is placed at the outer side of the forefoot. The patient is asked to resist the examiner's attempt to move the foot into dorsiflexion and inversion (Fig. 1.11). When weakness is present, the examiner needs to be aware of efforts to substitute the eversion movement by rotating the leg outwards at the hip.

Fig 1.11: Resisted eversion of the foot.

SENSORY CONDUCTION

The various areas are compared bilaterally at the same time (Fig. 1.12).

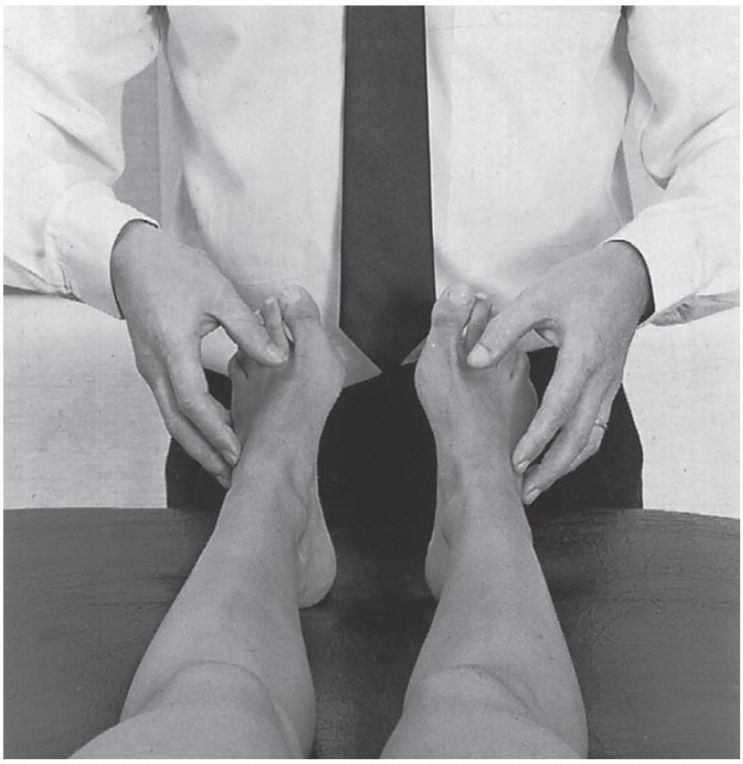


Fig 1.12: Testing for sensory conduction.

For the knee reflex text, each knee is raised in turn with one hand and the ligamentum patellae struck with the reflex hammer (Fig.1.13).

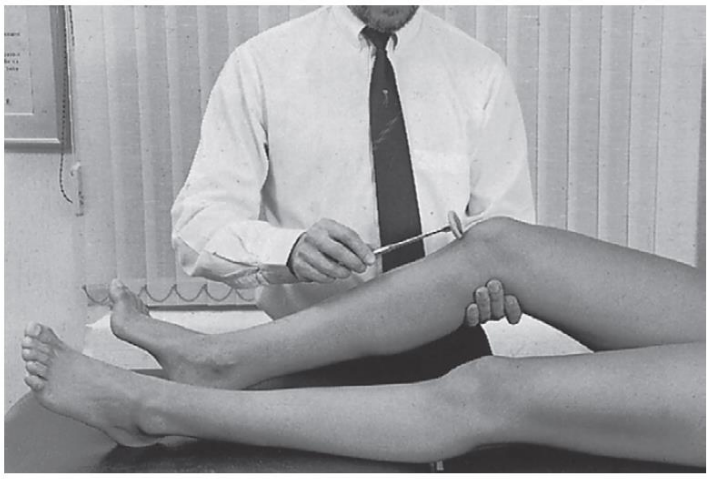


Fig 1.13: Knee reflex test.

In order to perfom the plantar reflex, the reverse end of the reflex hammer is run firmly over the plantar surface of the foot from the calcaneus along the lateral border to the forefoot, ending at the ball of the great toe (Fig. 1.14).

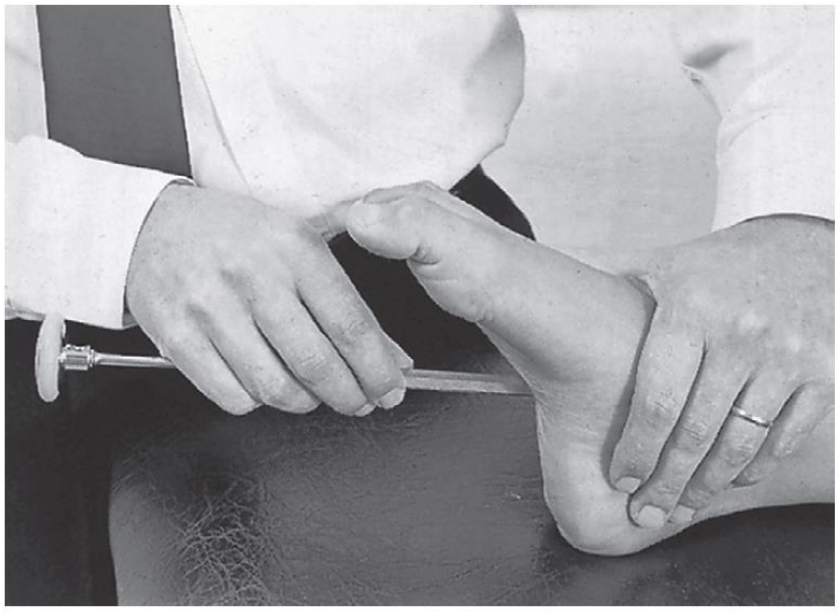


Fig 1.14: Plantar reflex test.

To test ankle reflex, the foot is raised with one hand. Then all the slack of the plantiflexors is taken up by the little finger pushing the foot into dorsiflexion, before striking the Achilles tendon (Fig.1.15).

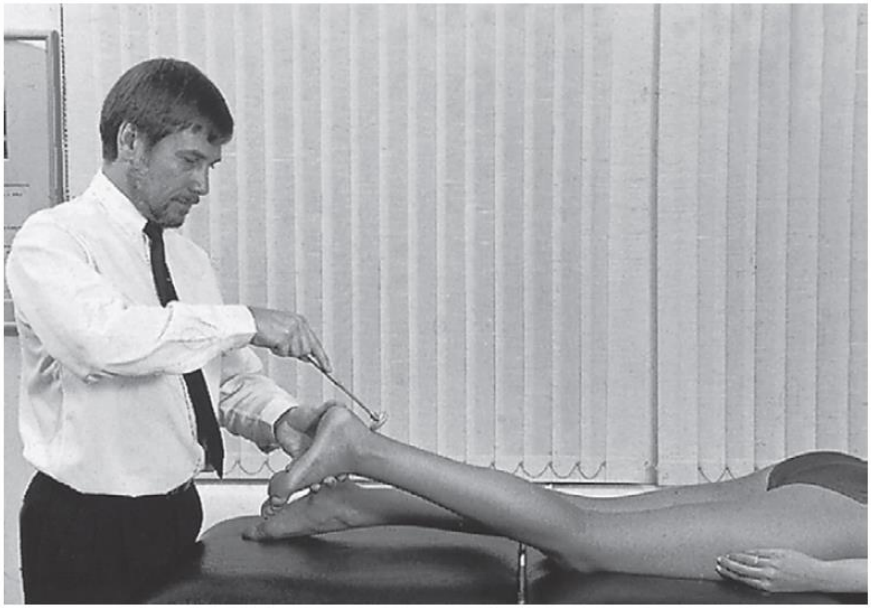


Fig 1.15: Ankle reflex test.

SUPINE EXAMINATION

PALPATION

To detect irregularities of the lumbar spinous processes, the index and middle fingers run quickly down the spine feeling for any abnormal projections (Fig. 1.16).

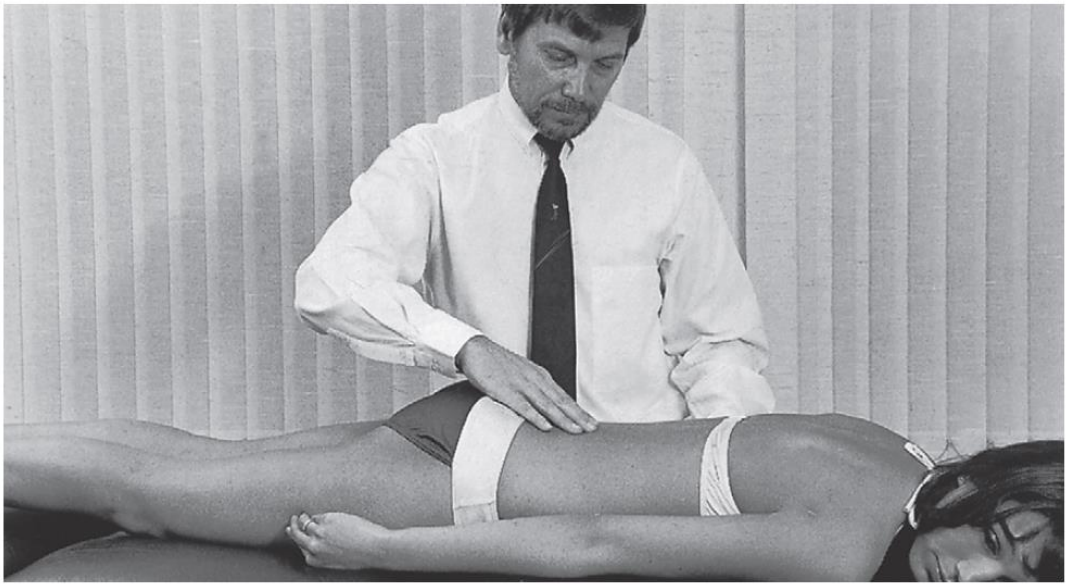


Fig 1.16: Palpation for irregularities of the spinous processes.

Next a series of pressures towards extension are exerted to detect the level of the lesion. Starting at the sacrum, each lumbar segment is 'sprung' in turn, and it should be noted at which level pain and muscle guarding are most provoked (Fig. 1.17).

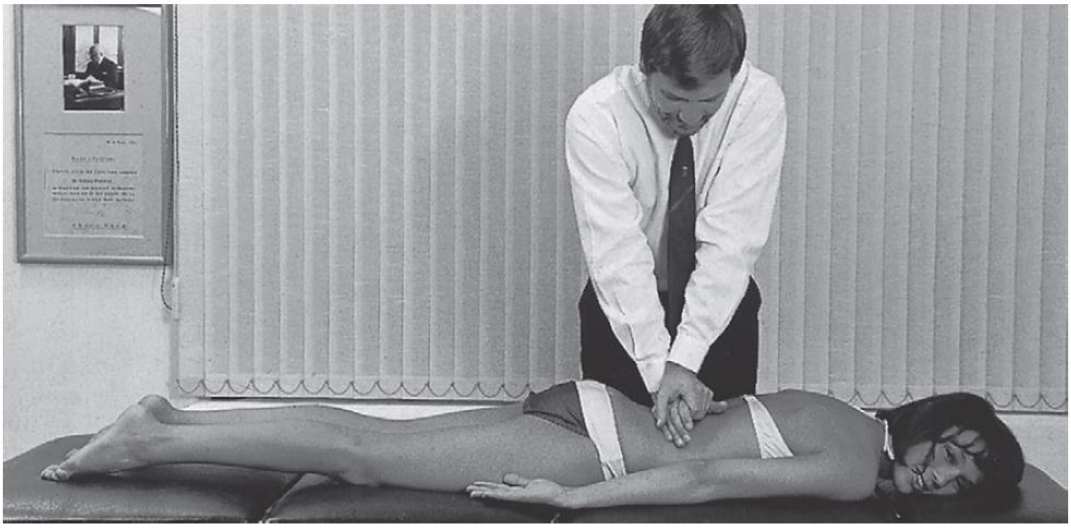


Fig 1.17: Pressures towards extension.

Annex 2
BIOLOGICAL SAMPLING

Partner-laboratory	FROM	Analyses	visit collection for clinical trial	sample type/ number		Collection (Y or N) and number of Year
UM-Ecellfrance	Patients : clinical centers	Immunomonitoring	Inclusion or V0, M1, M6	3 tubes de 10 mls hepariné 1 tube sec de 5 mls 1 tube PAXgene RNA (2,5 ml)		Yes, 5-10 years
	Patients : clinical centers	Anti HLA	V0, M6	serum 10 ml		Yes, 5-10 years
D Noel - u1183	Patients : clinical centers	miRNA	Inclusion or V0, M1, M6	serum 10 ml		Yes, 5-10 years
	Donor: production center (CytoSpin)	miRNA	V0	MSC	1 tube	Yes, 5-10 years
CNRS-STROMALAB	Donor: production center (CytoSpin)	Molecular profile	NA	Frozen cells end of production, 2,5	1 tube	Yes, 5-10 years
CNRS-STROMALAB	Donor: production center (CytoSpin)	Enzymology Test	NA	Frozen cells end of production, 3 millions/tube	1 tube	Yes, 5-10 years
CHU Nantes-INSERM 1229	Donor: production center (CytoSpin)	differentiation and potency assay	NA	Frozen cells end of production, 3 millions/tube	1 tube/donor	yes, 5-10 years
NUIG-REMEDI	Donor: production center (CytoSpin)	CELL-HOST INTERACTION	N/A	production, 2,5 OR 3 millions/tube	1 tube/donor	Yes, 5-10 years

Patients enrolled in RESPINE CLINICAL TRIAL will be informed about their biological samples going to be analysed at UM EcellFrance and to University of Montpellier (France)

Sampling from donors are not part of the RESPINE CLINICAL TRIAL but part of the RESPINE PROJECT. The donors have to be informed adequately and sign a specific consent form according to local lesgislation.

Supplementary Table 6. Donor Specific Antibodies (DSA) in patients (n=50) with degenerative disk disease before (M0), and three months (M6) after allogeneic bone-marrow derived mesenchymal stromal cells

Patients with preexisting DSA are indicated in pink (3 out of 50 patients) and patients developing de novo DSA are indicated in blue (5 out of 50 patients).

Patients 01-001 to 07-023

Patient number	Sex	HLA class I		HLA class II	
		M0	M6	M0	M6
01-001	M	neg	neg	neg	neg
01-004	M	neg	neg	neg	neg
01-005	M	neg	neg	neg	neg
01-011	M	neg	neg	neg	neg
01-012	F	neg	A*33 (10000) B*65 (5000)	neg	neg
01-013	M	neg	neg	neg	neg
01-018	M	neg	neg	neg	neg
01-021	F	neg	neg	neg	DQB1*05 (2900)
01-022	M	neg	neg	neg	neg
01-026	M	neg	neg	neg	neg
01-028	M	neg	neg	neg	nzg
01-032	F	neg	neg	neg	neg
02-001	M	neg	neg	neg	neg
02-003	M	neg	A*33 (14000) B*65 (9500) C*08 (2000)	neg	neg
02-006	M	neg	neg	neg	neg
03-006	M	neg	neg	neg	neg
04-005	M	neg	neg	neg	neg
04-008	F	neg	neg	neg	neg
05-001	F	neg	neg	neg	neg
05-004	F	neg	A*02 (20000)	neg	neg
05-007	F	neg	neg	neg	neg
05-008	F	neg	neg	DRB1*01 (4000) DQB1*05 (5000)	DRB1*01 (4000) DQB1*05 (5000)
05-013	F	neg	neg	neg	neg
05-014	M	neg	neg	neg	neg
06-006	F	A*01 (8000) A*11 (10000)	A*01 (10000) A*11 (12000)	neg	neg
06-010	M	neg	neg	neg	neg
06-012	M	neg	neg	neg	neg
06-015	M	neg	neg	neg	neg
06-018	M	neg	neg	neg	neg
06-021	M	neg	A*01 (11000) A*11 (9000)	neg	neg
06-022	F	neg	neg	neg	neg

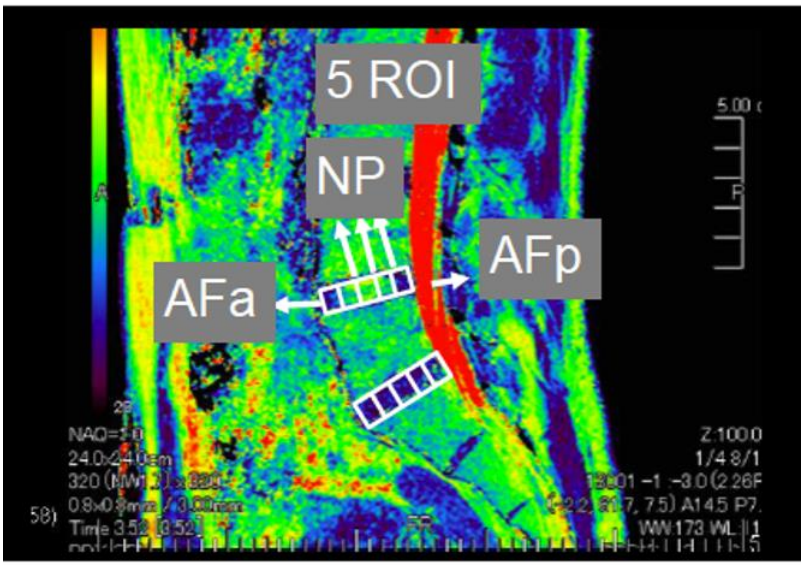
Supplementary Table 7. Donor Specific Antibodies (DSA) in patients (n=50) with degenerative disk disease before (M0), and three months (M6) after allogeneic bone-marrow derived mesenchymal stromal cells

Patients 07-001 to 09-028

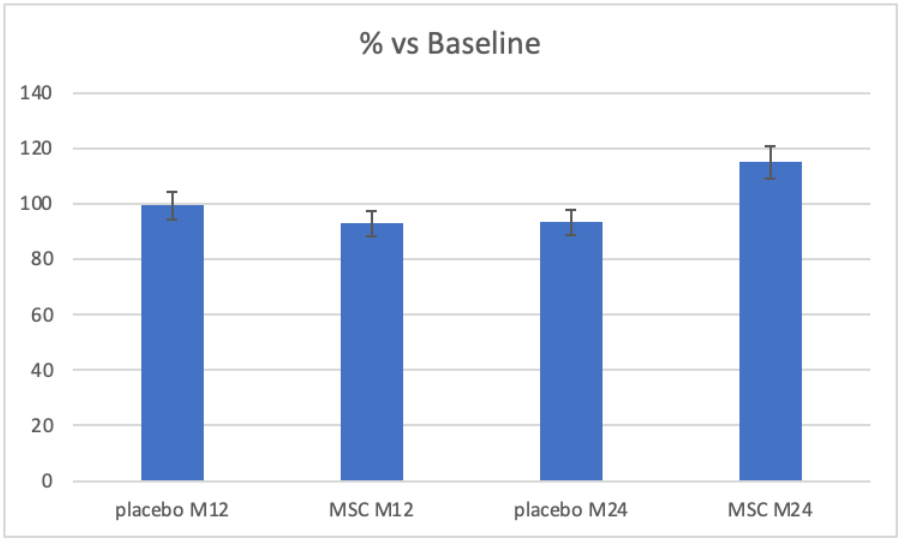
Patient number	Sex	HLA class I		HLA class II	
		M0	M6	M0	M6
07-001	M	neg	neg	neg	neg
07-003	M	neg	neg	neg	neg
07-006	F	neg	neg	neg	neg
07-008	M	neg	neg	neg	neg
07-010	F	neg	neg	neg	neg
07-011	M	neg	neg	neg	neg
07-014	M	neg	neg	neg	neg
07-015	F	neg	neg	neg	neg
07-018	M	neg	neg	neg	neg
07-021	M	neg	neg	neg	neg
07-023	F	neg	neg	neg	neg
08-003	M	neg	neg	neg	neg
08-007	F	neg	neg	neg	neg
08-011	M	neg	neg	neg	neg
09-004	M	neg	neg	neg	neg
09-008	M	neg	neg	neg	neg
09-011	F	A*11 (5000)	A*11 (10000)	neg	neg
09-021	F	neg	neg	neg	neg
09-028	F	neg	neg	neg	neg

Supplementary Table 8. Modification of lumbar MRI (Pfirrman score) at baseline and month 12 after treatment (BM-MSC or Placebo (P)) in both group (n=55)

patients	treatment	disc injected	pfirrman day 0	pfirrman M12
004	MSC	L5-S1	4	4
005	MSC	L5-S1	4	4
012	MSC	L5-S1	5	4
013	MSC	L5-S1	3	4
018	MSC	L5-S1	5	5
021	MSC	L3-L4	4	4
022	MSC	L5-S1	5	6
026	MSC	L5-S1	5	4
001	MSC	L5-S1	4	4
003	MSC	L5-S1	5	5
006	MSC	L5-S1	4	4
001	MSC	L5-S1	5	6
003	MSC	L5-S1	4	4
006	MSC	L4-L5	5	5
010	MSC	L3-L4	5	4
012	MSC	L4-L5	5	5
021	MSC	L4-L5	4	4
001	MSC	L4-L5	5	4
006	MSC	L4-L5	5	5
008	MSC	L5-S1	4	5
010	MSC	L5-S1	5	4
011	MSC	L5-S1	5	6
002	MSC	L4-L5	5	4
003	MSC	L5-S1	4	4
008	MSC	L5-S1	5	4
011	MSC	L5-S1	4	4
021	MSC	L5-S1	4	4
024	MSC	L3-L4	6	5
028	MSC	L5-S1	4	4
030	MSC	L4-L5	5	5
007	P	L4-L5	5	4
0010	P	L5-S1	4	5
014	P	L4-L5	4	4
020	P	L5-S1	5	5
023	P	L5-S1	5	4
027	P	L5-S1	4	4
030	P	L4-L5	5	5
008	P	L5-S1	5	4
012	P	L4-L5	5	5
004	P	L5-S1	6	7
005	P	L5-S1	5	4
014	P	L5-S1	6	5
017	P	L5-S1	5	5
019	P	L5-S1	5	4
002	P	L5-S1	4	4
004	P	L4-L5	5	4
005	P	L4-L5	4	5
012	P	L5-S1	4	4
013	P	L5-S1	5	4
017	P	L4-L5	6	5
020	P	L3-L4	4	4
001	P	L5-S1	5	5
022	P	L4-L5	5	6
027	P	L5-S1	5	6
031	P	L5-S1	5	5



Supplementary Figure 3 : example of Spin echo T2 sequences lumbar MRI and through analysis of 5 ROI



Supplementary figure 4 : Modification of lumbar MRI water content of T2 mapping on lumbar MRI (mean of 5 ROI on the disc) month 12 (=55) and 24 (n=20) compared to baseline after treatment (BM-MSC or Placebo) in both group