Disc_allo_MSV protocol. v.1.6 1 NCT01860417
CLINICAL TRIAL PROTOCOL:
Treatment of lumbar degenerative disc disease with allogeneic mesenchymal cells (MSV *). * MSV: "Ex Vivo" expanded bone marrow stromal mesenchymal cells with GMP procedure of the Institute of Molecular Biology and Genetics of Valladolid
Version: October 3, 2012
(Treatment of degenerative disc disease with allogenic mesenchymal cells MSV)
CLINICAL TRIAL PROTOCOL
Eudra-CT: 2012-004444-30
Code: Disc_allo_MSV
Title: Treatment of lumbar degenerative disc disease with allogeneic mesenchymal cells (MSV). * MSV: "Ex Vivo" expanded bone marrow stromal mesenchymal cells with GMP procedure of the Institute of Molecular Biology and Genetics of Valladolid
Development phase: Phase I – II
Version: 1.6, of October 3, 2012

Sponsor: Citospin, Scientific Park of the University of Valladolid R + D Building, Paseo de Belén, 11. Campus Miguel Delibes, 47011 Valladolid. NIF. B-47673538

LIST OF ABBREVIATIONS
AA Adverse event
AAG Serious adverse event
AEMPS Spanish Agency for Medicines and Health Products
GCP Good Clinical Practice (GCP)
GLP Good Laboratory Practice (GLP)
CEIC Ethical Committee for Clinical Research
CMN Mononuclear cells
CRD Data Collection Notebook
ECG Electrocardiogram
FTH Teófilo Hernando Foundation
DDL Lumbar Degenerative Discopathy
GMP Good Manufacturing Practice or Good Manufacturing Practice (NCF)
HCUVa Hospital Clínico Universitario de Valladolid
IBGM Institute of Molecular Genetic Biology, Uva
ICH International Conference on Harmonization
LSN Límite Superior Normal (Normal Upper Limit)
MO Bone Marrow
MSC Mesenchymal Cells
MSV Mesenchymal Cells of Valladolid (IBGM Procedure)
Non-steroidal Anti-inflammatory NSAIDs *
PCUVa Scientific Park of the University of Valladolid
PEI Research Product
SOPs Standard Operating Procedures
PRP Platelet Rich Plasma
ROI Region of Interest
QC Quality Control
SDV Source data verification
UVa University of Valladolid

* NSAIDs stands for “nonsteroidal anti-inflammatory drugs”, sometimes abbreviated as well NAIDs

INDEX:

1. SUMMARY

Type of request:
Phase I-II, single-center, prospective, randomized, blinded ** and controlled clinical trial for the treatment of lumbar degenerative disc disease with allogeneic mesenchymal cells (MSV *), with two arms with 12 patients each. In the experimental arm, cells will be administered locally in the nucleus pulposus area of the affected segment "Ex Vivo" expanded allogeneic mesenchymal cells with the IBGM GMP procedure (MSV; PEI No. 10-134; authorized by the AEMPS). The control arm will infiltrate the area of the paravertebral musculature close to the injury with saline solution containing mepivacaine at 1% in the same volume and subject to the same maneuvers as the intervention on the arm experimental.
** Double blind, masked for the patient and evaluators performing the analysis of the results, open to the doctor who applies the cells.

Data relating to the Sponsor:
Citospin,
Scientific Park of the University of Valladolid
R + D Building, Paseo de Belén, 11
Campus Miguel Delibes
47011 Valladolid
NIF: NIF. B-47673538
Title of the clinical trial:
Treatment of lumbar degenerative disc disease with allogeneic mesenchymal cells (MSV *). * MSV = Mesenchymal cells of the bone marrow prepared by the procedure of IBGM of Valladolid.

Protocol Code: Disc_allo_MSV
Eudra-CT: 2012-004444-30

Experimental and control drug: dose, pharmaceutical form, route of administration, therapeutic group:
Experimental drug: The investigational drug or product is MSV, which is Mesenchymal bone marrow stromal cells obtained from healthy donors with negative serology (NAT; nucleic acid technology) and processed according to procedure of the IBGM (PEI Num. 10-134; approved by AEMPS), with the following characteristics:
 Cell dose: 25x10e6 ± 5x10e6 MSV per disk.
 Presentation: 2 ml of cell suspension in Ringer-lactate isotonic with 0.5% human albumin and 5 mM glucose in a 5 ml syringe. Stability:> 12 hours at 4-20 ºC
 Administration: Injection into the nucleus pulposus area of the affected disc
 Therapeutic Group: Cell Therapy
Control drug: Injection of 2 ml of 1% mepivacaine in saline serum (see section 2.4). in the paravertebral musculature close to the injury, using the same volume and the same maneuvers as the intervention in the experimental arm.
Cell Production
Citospin, Cell Production Unit, Scientific Park of the University of Valladolid, R + D Building, Campus Miguel Delibes, Paseo de Belén, nº 11, 47011 Valladolid. Tel. 983184827. Technical Director: Dra. Ana García Sánchez
Phase of the clinical trial
Phase I-II
Objectives:
Main goal:
 Evaluate the feasibility and safety of the local use of allogeneic MSV for the treatment of lumbar degenerative disc disease.
Secondary objectives:

 Evaluate the efficacy of allogeneic MSV treatments using criteria of clinical evolution objectified by the low back pain questionnaires (Visual Scale Analog, VAS), disability (Oswestry questionnaire) and quality of life (questionnaire SF-36 in its abbreviated version, SF-12).
 Follow-up of the evolution of disc dehydration by the images of MRI calibrated at T2.
 Compare the efficacy of the treatments in the control and experimental arms.

Total number of patients
The number of patients treated will be 24, 12 for each of the branches.
Participating countries
The study will be carried out at the Hospital Clínico Universitario de Valladolid. Avda. Ramón y Cajal nº 3, 47003 Valladolid, Spain
Treatment duration
The implantation will be carried out in a single session and the evolution of the patients for 12 months.
Schedule and expected completion date
Start of study: last quarter of 2012
Recruitment: for a maximum of 6 months, until 12 patients are obtained for each branch.
Duration of the trial: 12 months after the treatment of the last patient.

2. JUSTIFICATION OF THE TEST AND ITS DESIGN

2.1. ABSTRACT

Intervertebral disc degeneration is a very common disease, which can lead to origin to low back pain, radiculopathy or myelopathy. The combination of physical treatment and The doctor solves 90% of the cases, but 10% becomes chronic, generating a serious health problem, since chronic low back pain spoils both the quality of life of the patient as their work capacity, with an enormous economic impact on the System of Health. Chronic cases often require surgery, which usually relieves pain, but gives lead to adverse effects on mobility and accelerate degeneration of the segments adjacent vertebral bodies. We have recently published the results of the pilot study with our autologous mesenchymal cells (MSV), demonstrating its feasibility and safety, and remarkable clinical efficacy (71% of optimal effect after 12 months), which compares favorably with surgical treatment (arthrodesis or disc replacement), but MSV treatment is much simpler and more conservative (Orozco et al., 2011). Yes If it were possible to use allogeneic MSVs, it would be greatly simplified and the cost of treatment, considerably increasing the number of patients who could benefit from it. On the other hand, data from the literature show that mesenchymal cells, due to their low antigenicity and immunomodulatory effect, escape to antigen recognition and are tolerated by the recipient's immune system. This Tolerance has been extensively studied in animal models and, more recently, has been has demonstrated the feasibility and safety of mesenchymal cell treatments allogeneic in humans, with the completion of several published phase II trials and several dozens declared in the NIH database in the recruitment or implementation phase. Us We ourselves have an allogeneic MSV trial underway, exactly the same product proposed here, for the treatment of knee osteoarthritis (Eudra-CT: 2011-005321-51). In the present proposal, a prospective phase I-II clinical trial is proposed. randomized, blinded and controlled, with 2 arms of 12 patients each.
In the arm experimental mesenchymal cells obtained from healthy donors purified and expanded under GMP conditions using our protocol, authorized by the AEMPS (MSV, PEI No. 10-134). MSVs will infiltrate the affected intervertebral discs. Whenever possible (because there is sufficient material and explicit consent), use surplus cells from patients undergoing MSV therapy as donors autologous, which would significantly simplify the procedure. In the control arm it will infiltrate the area of the paravertebral musculature near the injury with saline solution containing 1% mepivacaine in the same volume and subject to the same maneuvers as the intervention in the experimental

arm. Study participants will be offered the possibility of receiving MSV treatment once the study is completed if it is demonstrated beneficial and the patient so wishes. The application of the cells will be carried out in the Valladolid University Clinical Hospital. The evaluation of the response to treatment will be blind and will be followed by the evolution of the indices of low back pain, disability and quality of life, as well as the improvement of the dehydration of the treated segments, quantified from magnetic resonance images (T2 sequence).

2.2. EPIDEMIOLOGY OF DEGENERATIVE DISC DISEASE. REPERCUSSION ECONOMIC AND SOCIAL.

Intervertebral disc degeneration is a very common disease, seen in 80% of the subjects older than 60 years. Most of these individuals are asymptomatic, but in some of them it can lead to axial skeletal pain, radiculopathy and myelopathy. Combining physical and medical therapies is successful in relief of pain in about 90% of cases. However, the remaining 10% is chronic and generate a serious public health problem, since chronic low back pain ruins both the quality of life and the work capacity of the patient and increases the use of health services (Errico et al., 2004; Balague et al., 2007). Chronic low back pain is one of the most prevalent pathologies in the countries industrialized: 75% of the population has suffered it at some time and, although 90% are cured In 3 months, 10% become chronic, which represents 5-6 million people in Spain and 67 million in the EU (http://www.zimmer2.eu/lancs/incidence_backpain.html). CL is a very important cause of absenteeism, which is required for 59% of the patients. It is estimated that it is the second in importance, with 700,000 days / year in the EU. It also promotes an overuse of the health system, both in the use of physical means (for example RX), as in medication (90% of patients use drugs). On the whole, It is estimated that it represents 1-3% of health spending in the EU (http://www.zimmer2.eu/lancs/incidence_backpain.html). In Spain it is estimated that an expense of about 6,000 million euros per year (Robaina, 2006). The intervention surgery (usually arthrodesis), which is applied to about 1000 patients per year in Spain is the "gold standard" to solve the problem of pain, but it is an expense considerable and is not free of side effects, so its systematic use is a highly controversial topic (Errico et al., 2004; Airaksinen et al., 2006; Kwon et al., 2006; Harrop et al., 2008).

2.3. PATHOPHYSIOLOGY OF THE INTERVERTEBRAL DISC (IVD) AND DISCOPATHY LUMBAR DEGENERATIVE (DDL).

The intervertebral disc (IVD) is made up of two basic components, the annulus fibrosus (AF) and the nucleus pulposus (NP), separated by a transition zone. The fibrous ring The outer layer is made up of about 25 layers of type I collagen (70-80%) that are oriented following an angle of 60º between adjacent vertebrae, and by cells of the fibrocyte / fibroblast arranged concentrically with a density of 9x106 cells / cm3. The non-elastic collagen fibers of each layer insert into the bodies adjacent vertebral bodies. In the transition zone the fibrosites are being replaced by chondrocytes and type II collagen, elastic, by type I collagen, highly resistant to distention (Eyre & Muir, 1976; Nerlich et al., 1998) NP contains collagen (80% type II), notochord-derived cells similar to chondrocytes and polyglycans. Its composition is similar to that of hyaline cartilage although with more polyglycans and a different proportion of them. It has no vascularity and the number of cells per unit volume is very small, about 4x106 cells / cm3 (Roughley, 2004; Zhao et al., 2007). NP cells are adapted to live at low oxygen tension, low glucose concentration and low pH (Urban et al., 2004; Bibby et al., 2005). In fact, it has been proven "In Vitro" that very low oxygen levels induce the differentiation of mesenchymal cells towards cell-specific phenotypes nucleus pulposus (Horner & Urban, 2001; Bibby et al., 2005).

Collagen fibers confer tensile strength and proteoglycans are responsible for the ability to retain water and ultimately the firmness and compressive strength. The water content varies depending on the external load to which DIV is submitted. In high load situations the water is squeezed into the saucers adjacent vertebral bodies (endplates,

containing a 1 mm layer of cartilage thickness) and instead returns to the disc in low load situations. For this mechanism works it is essential that the nucleus pulposus maintains its hydrophilic nature, It depends mostly on proteoglycans. The mechanism of water exchange through of the end plates is necessary for the maintenance of the PN, which is avascular, since that nutrients and waste products are incorporated and removed by diffusion to and from the vessels of the outer ring and vertebral body (Sztrolovics et al., 2002). The End plates also act as physical barriers to prevent core intrusion pulposus in the trabecular bone of the vertebral bodies. The vascularization and innervation of nondegenerate IVD is limited to the external fibrous ring at through the sinuvertebral nerve, which innervates the posterior and posterolateral regions and the gray branch, which is mainly distributed along its lateral and anterior areas. The nucleus pulposus has no innervation (Coppes et al., 1990). In degenerated discs, the number of nerve fibers and it is possible that cytokines produced in the NP reach, at through fissures produced in the AF, the nerve endings, producing their irritation chemistry (Aoki et al., 2002).

The IVD distributes mechanical forces through the column, preserving its stability and giving it flexibility. From the age of 30 it is normal for radiology to show signs initials of vertebral osteoarthritis at some level of the spine. When the NP ages it is reduced the number of chondrocytes and the concentration of proteoglycans, and the disc is dehydrated with the consequent alteration of its physical properties, transforming from mucoid to fibrous and rigid. In the fibrous ring, the fibrillar organization deteriorates, forming channels that allow the contents of the NP to herniate through the ring. If degeneration disc is gradual, generally painless, and radiological signs can be seen without clinical translation. If the process develops abruptly, many times As a consequence of over-requests, it tends to give clinical symptoms. Possibly, the decreased damping capacity could facilitate the triggering of the neurological mechanism that can trigger pain. In conclusion it is thought that the NP is, in many cases, the starting point of the degenerative cascade and, therefore Consequently, it should be a priority point of therapeutic attention (Adams et al., 2000).

2.4. CURRENT TREATMENTS FOR DEGENERATIVE DISCOPATHY.

Disc degeneration has different clinical translations. May lead to herniation disc material and, depending on the location, generate symptoms ranging from isolated low back pain and / or associated with radiculopathy, myelopathy or claudication neurogenic disease associated with canal stenosis. According to the degree of invasion of the structures next we speak of protrusion or hernia. On many occasions, however, there is pain lumbar without herniation and with integrity of the fibrous ring. It is in these cases when proposes the treatment described in this RC.

The first step of treatment includes physical measures for stabilization of the lumbar spine. Both by external means (girdles or corsets), and, mainly, by toning the abdominal and spinal muscles through exercises isometric that do not require mobilization of the lumbar spine. As a complement of these measures the weight will be controlled to an adequate level and measures will be implemented of postural hygiene: by using ergonomic chairs and furniture, avoiding flex the spine forward and keep the weights close to the body. The use of both non-steroidal anti-inflammatory drugs such as corticosteroids, analgesics and the Muscle relaxants may be helpful in periods of exacerbation of pain. The application of currents, massages, tractions and heat in rehabilitation and acupuncture can improve symptoms temporarily. The next step includes periarticular infiltrations of local anesthetic and corticosteroids, which can serve both as a diagnosis of the origin of the pain and treatment of the acute phase. The final step is surgery, in which the lumbar spine is stabilized by fixing the affected vertebrae. Surgical treatment is necessary when the condition clinical is intense and refractory to drug and rehabilitation treatments. The discectomy associated or not with vertebral segment arthrodesis is the “gold standard” and its efficacy is beyond question (Errico et al., 2004; Kwon et al., 2006). Fixing is done with the patient's own bone graft and is facilitated with metal implants. On discs degenerates expressed as a protrusion

or contained herniated disc can be considered percutaneous nucleotomy performed under fluoroscopic control, either mechanical, electrothermal or chemical (chymopapain, DiscoGel). Surgical interventions usually have very satisfactory analgesic effects, but are inevitably iatrogenic, reducing mobility and destabilizing neighboring segments, even when these effects are sometimes subclinical (Hilibrand & Robbins, 2004; Harrop et al., 2008; Rihn et al., 2009).
The interest in preserving the mobility of the affected segment led to the development of prostheses of disk. In the early stages of disc degeneration, nucleus pulposus implants, they can occupy the therapeutic space between discectomy and arthrodesis. Ideally, they are capable of recovering the turgor of the disc, the tension of the fibrous ring and the ability to distribute loads evenly across the disc space. Most designs use viscoelastic polymers that are injected into the disc space (Strange & Oyen, 2012).
In Figure 1 we compare the therapeutic efficacy of different procedures in seven recent high-quality clinical trials. Data on the evolution of pain and disability have been calculated and expressed on a scale of 0-100% and the comparison of The treatments have been carried out by comparing the slope of the line that relates relief of the pain versus initial pain (Huskisson, 1974). The slope of the line defines the efficiency of any particular treatment, this maximum being when a slope of 1 (Total relief, Figure 1). Details of the analysis can be found in the publication original (Orozco et al., 2011). Exercise, conventional "non-surgical" treatment plus Common, it was the least potent treatment, with an efficacy of 0.07 (Fritzell et al., 2001). The efficacy of treatments involving cognitive intervention and exercise ranged between 0.20 and 0.29 (Brox et al., 2003; Fairbank et al., 2005; Brox et al., 2006), and the protocols of spinal fusion were the most effective, with an efficacy ranging from 0.30 to 0.71 in different trials (Fritzell et al., 2001; Brox et al., 2003; Fairbank et al., 2005; Hohaus et al., 2008; Guyer et al., 2009). Disc replacement protocols with artificial discs had the same efficacy as fusion alone (Guyer et al., 2009; Siepe et al., 2009). Finally, In one trial, the effects of discectomy were compared to discectomy plus treatment with expanded cells in the excised disc material (ADCT in Figure 1) a small but significant improvement was found in this group, although only after 2 years (Hohaus et al., 2008). This figure also represents the results obtained recently by us with autologous MSVs (Dotted curve marked “This study”) (Orozco et al., 2011). The efficacy reached a value of 0.71, a value indistinguishable from the maximums reached with surgical treatments (see discussion in the section next ahead).

2.5. BACKGROUND OF CELLULAR THERAPY.
Advances in understanding the pathophysiology of degenerative disc disease have promoted the study of new biological therapies, including cell therapy. The Potential advantages of these treatments are the preservation of anatomy, biomechanics, and the normal movement of the areas near the injury. Cell therapy, especially with mesenchymal cells (MSC), has produced interesting results in both in vitro and in vivo (Fassett et al., 2009). Co-culturing MSC with extracted cells nucleus pulposus (NP) stimulates the proliferation of nucleus pulposus cells and the differentiation of MSCs towards the chondrogenic lineage (Vadala et al., 2008; Yang et al., 2008; Le Maitre et al., 2009; Watanabe et al., 2009). Increased production of cytokines, in particular TGFβ, favors these transformations (Risbud et al., 2004; Yang et al., 2008; Yang et al., 2010). The nucleus pulposus contains MSCs that are very similar to the MSCs obtained from bone marrow (Blanco et al., 2010), and studies in animal models of disc degeneration have shown that MSCs injected into the nucleus area pulposus survive for months, and even proliferate in canine models (Hiyama et al., 2008; Blanco et al., 2010), porcine (Henriksson et al., 2009), and rabbit (Sakai et al., 2005). In addition, the transplanted MSCs induced the production of matrix proteins. extracellular, including aggrecan and other proteoglycans, and types I and II collagen (Sakai et al., 2005; Hohaus et al., 2008; Henriksson et al., 2009). Finally, these studies of the height and water content of the disc (Sakai et al., 2005; Hiyama et al., 2008; Hohaus et al., 2008).

We recently conducted a pilot study in humans with autologous MSCs (Orozco et al., 2001). Our results demonstrated the feasibility and safety of the procedure and indicated an efficacy close to 71%, which compares favorably with other procedures (Figure 1). A small rehydration was also documented but significant number of affected discs after one year of treatment (Orozco et al., 2011). The procedure is very attractive, as it equals in efficacy to arthrodesis, the gold-standard of current therapeutic procedures, but it is much more conservative, preserves the normal biomechanics and does not require surgery or hospitalization of the patient. The use of cells However, autologous makes the procedure very time consuming and expensive.
The use of allogeneic cells in cell therapy procedures would expand noticeably its horizon, since it would simplify the procedure, improve the yields and would lower costs, making treatment accessible to much broader layers of the population. Disadvantages include the possibility of rejection by the immune system, as is well known in the case of transplants. In the case of MSCs we have, without However, a favorable circumstance, and it is the repeatedly proven fact that these cells evade antigen recognition and, furthermore, inhibit responses immune in a way not restricted by the HLA system, leading to tolerance immunological (Le Blanc et al., 2003; Aggarwal & Pittenger, 2005; Jiang et al., 2005; Klyushnenkova et al., 2005; Sotiropoulou et al., 2006; Le Blanc & Ringden, 2007; Sotiropoulou & Papamichail, 2007). In animal experimentation, repeatedly allogeneic MSCs without any indication of rejection or immune problems later. Circumscribing the evidence to studies regarding the regeneration of cartilage, we have examples in rabbit models (Yan & Yu, 2007; Miyamoto et al., 2010; Dashtdar et al., 2011) and pig (Shimomura et al., 2010; Chang et al., 2011). The safety of allogeneic mesenchymal cells has recently been shown also in humans, where the results obtained in two clinical trials for the treatment of systemic lupus erythematosus, no adverse effects after administration endovenous (Sun et al., 2009; Liang et al., 2010). Results have recently been obtained similar with cord blood derived allogeneic mesenchymal cells (Sun et al., 2010).
There are also numerous treated cases of breast cancer patients (Koc et al., 2000), graft versus host disease (Lazarus et al., 2005), and other diseases autoimmune (Bernardo et al., 2009; Bernardo et al., 2011) without there having been, until now, adverse effects. Finally, there are declared 69 clinical trials using MSC allogeneic in the database "clinicaltrials.gov" for the treatment of very pathologies varied, including not just graft versus host disease and different autoimmune diseases, but also ischemic strokes of the brain, heart or peripherals, liver destruction, osteogenesis imperfecta, or disc degeneration intervertebral (see http://clinicaltrials.gov/ct2/results?term=mesenchymal+allogenic). On summary, both the experience of animal experimentation and use in trials Clinical trials in humans demonstrate the safety of allogeneic mesenchymal cells, without adverse effects have been reported. These antecedents, together with the notable benefits for patients that its use would bring fully justify its use in the proposed essay.

2.6. PROPOSED ALTERNATIVE TREATMENT.

Based on all of the above, we propose a clinical trial with two arms:
1) Experimental group: treatment with allogeneic MSV from donors, with informed consent, isolated and expanded as described in our protocol (PEI No. 10-134; see details below). Expand following the GMP procedure until obtaining 25x106 cells (about 3 weeks), that are processed and managed as detailed below.
2) Control group: Injection in the area of the paravertebral muscles close to the lesion of saline serum containing 1% mepivacaine in identical volume and subject to the same maneuvers as the intervention in the experimental arm.
The proposed treatment is based on our own previous trial, using Autologous MSVs (Orozco et al., 2011), but in this case we propose to use cells allogeneic, which would simplify and make the procedure cheaper, making it much more universal. Previous experience with allogeneic MSCs (including our own experience in the ongoing Eudra

CT 2011-0053221-51) has shown no adverse effects, even in parenteral administration circumstances (see below)

2.6.1 General characteristics of the product under study. The investigational drug or product (experimental arm) is MSV, which is bone marrow mesenchymal cells processed according to the IBGM system of Valladolid, suspended in isotonic Ringer-lactate medium with 0.5% human albumin and 5 mM glucose. Cell dose: 25x106 ± 5x106 MSV, in a volume of 2 ml of cell suspension. Stability demonstrated is> 12 hours at 4-20 ºC. The whole process of obtaining MSV is carried out by highly qualified specialists in crops cell phones and is developed in the Cell Production Unit of Citospin (Parque Científico de la UVa), meeting GMP criteria and following protocols approved by the AEMPS (PEI No. 10-134).

In the validations carried out, MSV has been shown to possess the characteristics typical MSC defined by the Society for Cellular Therapy (Dominici et al., 2006): 1) Adherence to plastic in cultivation; 2) Expression of CD73, CD90 and CD105 antigens. 3) Absence of hematopoietic antigens, markers of monocytes, macrophages and B lymphocytes. 4) In vitro differentiation capacity in osteoblasts, adipocytes and chondrocytes. Watch details in Figure 2 and Figure 3.

2.6.2. Obtaining, cultivation and expansion and application of MSV.

These matters are described in the section dedicated to the treatment, product of research and study procedures, sections 6.1.2 to 6.1.4.

2.6.3. Possible complications derived from the proposed therapy. Complications are similar to those of discography, and they occur very rarely. The most common complaint is exacerbation of pain for 1-2 weeks, which usually resolved by analgesia and muscle relaxants for a period short. The most serious complication is discitis, the incidence of which is less than 0.1% (Osti et al., 1990). Other complications described, although much less frequently, are transient headache, nausea, meningitis, epidural abscess, arachnoiditis, bruising intrathecal, retroperitoneal hematoma, cauda equina syndrome and acute disc herniation. Isolated cases of rash are not considered in this trial, as they are attributable to contrast medium. Initial fears about possible long-term discography side effects regarding disc viability have not been confirmed.

In a study with up to 20 years of clinical follow-up, no radiographic evidence of disc degeneration was found progressive after discographies (Flanagan & Chung, 1986). The possible risk of complications derived from the use of allogeneic cells has been discussed in detail above. Suffice now to summarize that, according to the literature, the application of Allogeneic MSCs, both locally and systemically, have been shown to be safe both in the context of clinical trials and studies in animal models. Either way, will monitor skin and mucosal reactivity to watch for a possible immune reaction.

2.7 BIBLIOGRAPHICAL BACKGROUND SUPPORTING SAFETY AND EFFECTIVENESS THERAPY.

Studies in animal models of disc degeneration have shown that MSCs injected into the nucleus pulposus area survive for months and even proliferate in the canine model (Hiyama et al., 2008; Blanco et al., 2010), porcine (Henriksson et al., 2009), and rabbit (Sakai et al., 2005). In addition, the transplanted MSCs induced the production of extracellular matrix proteins, including aggrecan and other proteoglycans, and collagen types I and II (Sakai et al., 2005; Hohaus et al., 2008; Henriksson et al., 2009). Finally, these studies also showed evidence of therapeutic efficacy, since the injection of the MSC resulted in a better preservation of the height and water content of the disk (Sakai et al., 2005; Hiyama et al., 2008; Hohaus et al., 2008). Very recently, we have conducted a pilot study in humans with autologous MSCs. Our results demonstrated the feasibility and safety of the procedure and indicated an efficacy close to 71%, which compares favorably with other procedures (Figure 1). In addition, a small but significant rehydration of the affected discs after one year of treatment (Orozco et al., 2011). The process is very attractive, as it equates in efficacy to arthrodesis, the gold standard of procedures current therapeutics, but it is much more conservative, preserves normal biomechanics and it does not require surgery or hospitalization of the patient. The use

of autologous cells does, without However, the procedure is very time consuming and expensive, which makes it highly desirable investigate the possibility of allogeneic cells. In an ongoing clinical trial (Eudra CT 2011-005321-51; NCT01586312) we are already testing the effects of allogeneic MSV, with good preliminary results. The study proposed here, studying allogeneic MSV as a therapeutic alternative to degenerative disc disease and including a control group, which considerably increases the power of the trial with respect to the previous one (Orozco et al., 2011) for two reasons: 1) The possibility of comparison with a controlled group, and 2) The potential versatility added by include a group with allogeneic cells. This last aspect deserves additional comment, which is developed in the next paragraph.

The use of allogeneic cells in cell therapy procedures would expand noticeably its horizon, since it would simplify the procedure, improve the yields and would lower costs, making treatment accessible to much broader layers of the population,. Disadvantages include the possibility of rejection by the immune system, as is well known in the case of transplants. In the case of MSCs we have, without However, a favorable circumstance, and it is the repeatedly proven fact that these cells evade antigen recognition and, furthermore, inhibit responses immune in a way not restricted by the HLA system, leading to tolerance immunological (Le Blanc et al., 2003; Aggarwal & Pittenger, 2005; Jiang et al., 2005; Klyushnenkova et al., 2005; Sotiropoulou et al., 2006; Le Blanc & Ringden, 2007; Sotiropoulou & Papamichail, 2007). In animal experimentation, repeatedly allogeneic MSCs without any indication of rejection or immune problems later. Circumscribing the evidence to studies regarding the regeneration of cartilage, we have examples in rabbit models (Yan & Yu, 2007; Miyamoto et al., 2010; Dashtdar et al., 2011) and pig (Shimomura et al., 2010; Chang et al., 2011). The safety of allogeneic mesenchymal cells has recently also been demonstrated in humans, where the results obtained in two clinical trials for the treatment of systemic lupus erythematosus, no adverse effects after administration endovenous (Sun et al., 2009; Liang et al., 2010).

Results have recently been obtained similar with cord blood derived allogeneic mesenchymal cells (Sun et al., 2010) .There are also numerous treated cases of patients with breast cancer (Koc et al., 2000) graft versus host disease (Lazarus et al., 2005), and other diseases autoimmune (Bernardo et al., 2009; Bernardo et al., 2011) without there having been, until now, adverse effects. Finally, there are declared 69 clinical trials using MSC allogeneic in the database "clinicaltrials.gov" for the treatment of very pathologies varied, including not just graft versus host disease and different autoimmune diseases, but also ischemic strokes of the brain, heart or peripherals, liver destruction, osteogenesis imperfecta, or disc degeneration intervertebral (see http://clinicaltrials.gov/ct2/results?term=mesenchymal+allogenic). On summary, both the experience of animal experimentation and use in trials Clinical trials in humans demonstrate the safety of allogeneic mesenchymal cells, no adverse effects have been reported. These antecedents, together with the notable benefits for patients that its use would bring fully justify its use in the proposed essay.

2.8 EXPERIENCE OF THE RESEARCH TEAM.

The research team has experience in both cell production and preclinical and clinical research with mesenchymal cells, with a run of several years of preclinical-clinical collaboration in research projects. The team will be coordinated by Dr. Javier García-Sancho, who has been the coordinator of the Cell Therapy Network of the Carlos III Health Institute (TerCel) for 8 years and that directs the Valladolid node, of the that depends on the Cell Production Unit for clinical use and the EBT Citospin. The doctor. García-Sancho has experience in managing research in Cell Therapy, both through preclinical and clinical level, and in the regulatory aspects of it. Has participated in numerous clinical trials. He is the corresponding author of the publication describing the use of autologous MSVs for the treatment of lumbar degenerative disc disease (EudraCT 2008-001191-68) (Orozco et al., 2011), and is the coordinating investigator

of a trial in March with allogeneic MSV for the treatment of knee osteoarthritis (MSV_Allo; Eudra-CT: 2011-005321-51; NCT01586312). The Cell Production Unit of the IBGM, according to GMPs, is directed by Dr. Ana Sanchez. It was the first Clean Room authorized by the AEMPS in the Public System of Health and is currently participating in six clinical trials, several of them with cells mesenchymals of the bone marrow. It has an MSC preparation protocol authorized by the AEMPS (PEI No. 10-134, Valladolid mesenchymal cells; MSV). It has a stable staff trained in quality control, which includes 12 people. It has recently given rise to a Technology-Based Company owned by the University, Citospin S.L. which adds additional nuances to cell production and counts with new and modern facilities in the Science Park of the University of Valladolid. Doctors Mercedes Alberca and Verónica García Díaz have more than 3 years of experience as Production Managers in our GMP room. The University Clinical Hospital team led by Dr. David Cesar Noriega Gonzalez, from the Traumatology and Orthopedics Service has extensive clinical experience in this specialty, especially in degenerative disc pathology with more than 10 years of dedication. It has been incorporated into preclinical and clinical studies of Cell Therapy in collaboration with the Valladolid Node of the TerCel Network within the framework of the Network Regenerative Therapy of Castilla y León. Dr. Ardura has 7 years of experience in the practice of the specialty.

3. OBJECTIVES

In this prospective study that will evaluate the feasibility and safety of local implantation in the nucleus pulposus area of allogeneic MSVs for the treatment of discopathy lumbar degenerative. The working hypothesis contemplated pursues the achievement of long-lasting analgesia and anti-inflammation and regeneration of degenerated IVD in degree enough to be objectifiable by questionnaires and image scans. The approach as a randomized and controlled trial will allow a study preliminary efficacy compared to the control arm,

3.1. Main goal

 To evaluate the viability and safety of allogeneic MSV, applied by percutaneous injection in the nucleus pulposus area for the treatment of lumbar degenerative disc disease, verifying that each of the the procedures established in the protocol and recording the possible adverse effects related to cell therapy and those that may arise for the duration of the clinical trial, whether or not related to the same.

3.2. Secondary objectives

 To determine clinical outcomes and image variations
Radiographic and Magnetic Resonance, which denote anti-inflammation, regeneration or, on the contrary, progression of lumbar IVD deterioration, after 12 months after the implementation of MSV in the NP.
 Compare the efficacy of MSV treatment with that obtained in the arm control.

3.3. Primary Criteria

3.3.1. Radiographic criteria

 In profile radiography, carried out with the same technical characteristics and hourly (before noon), the height of the space will be measured intervertebral in millimeters, this being the variable that will be compared with measurements that occurred over time (6 and 12 months after implantation of MSV). An increase in height will be understood as rehydration disc associated with an increase in proteoglycans and cellularity of the NP.

3.3.2 Magnetic Resonance Criteria

 A quantitative assessment of the water content of the affected segments on T2 images (Sakai et al., 2006; Hiyama et al., 2008; Watanabe et al., 2009), following the procedure below illustrated in Figure 4 (see foot for details) (Orozco et al., 2011). The initial values will be compared with those obtained 6 and 12 months after the intervention.

3.4. Secondary criteria

 Clinical observations of the surgeon
 Evaluation of lumbar pain and general health status according to the Visual Scale Analog (EVA, see Annex V). The score will be transformed to a scale 0-100% for analysis (Huskisson, 1974)
 Disability assessment using the Oswestry questionnaire (Davidson & Keating, 2002) (ODI, see Annex VI).
 Assessment of quality of life using the simplified version of the questionnaire Short Form-36 (Davidson & Keating, 2002). It has been shown that this simplification (SF-12; see Annex VII) has a statistical value that approximates much the complete test (Gandek et al., 1998).

4. TYPE OF CLINICAL TRIAL AND DESIGN OF THE SAME

4.1 Type of CT

Phase I-II clinical trial, single-center, prospective, randomized, blinded **, controlled for the treatment of lumbar degenerative disc disease with intact fibrous ring proven by MRI, with two arms: a control arm with placebo treatment (injection of mepivacaine at 1%) and another experimental arm treated with allogeneic MSV. This is the third application of a PEI consisting of Ex vivo expanded bone marrow mesenchymal cells (PEI No. 10-1349). In both cases, the product will be administered in a volume of 2 ml per injection in the nucleus pulposus area.
** Double blind, masked for the patient and evaluators performing the analysis of the results, open to the doctor who applies the cells.

4.2 Overall study design

The study consists of 2 arms, one experimental with allogeneic MSV, and another control in the to be injected into the area of the paravertebral muscles near the injury saline serum containing 1% mepivacaine (1 ml of 2% mepivacaine + 1 ml of saline) in identical volume and subject to the same maneuvers as the intervention on the arm experimental. Participants in the study will be offered the possibility of, once completed the study, receive MSV treatment if it proves beneficial and the patient so wishes. The inclusion criteria in both arms are identical, but after recruiting patients will be randomly assigned to one of the treatments. The Cell manufacturing will be carried out by Citospin at the facilities of the Park Scientist of the UVa and the application and monitoring will be carried out at the Clinical Hospital University of Valladolid.
24 patients diagnosed with low back pain and / or low back pain attributable to disc degeneration and evidence of proven continuity of the external fibrous ring in the magnetic resonance image. The patients will be evaluated clinically, by the previous imaging studies (X-ray and MRI). If they are considered inclusive, they will be provided with the information regarding the clinical trial and the "Patient Information Sheet" (Annex II), citing them for the Inclusion visit. In the "Inclusion Visit", if the patient decides to participate in the trial, they must sign the "Informed Consent Document" and a visit will be scheduled for the practice of MRI and radiographs, which will be considered the pattern on which the possible determinable changes in the controls at 6 and 12 months. If you are MRI and X-rays have been recently performed and their characteristics are valid for the study, its repetition can be avoided. In this visit, basic analytics will also be requested, serology for HIV, lúes and hepatitis B and C, and a serum sample will be obtained that will be sent to the Cell Production Unit for filing in the Seroteca. Although the Positive serology is not a criterion for exclusion of the recipient in the present test, it will be performed for epidemiological and preventive purposes. At the next visit (V0), it will be verified that all the inclusion criteria persist and that no exclusion is presented and the patient will be randomized (blind for him) in one of the two treatment groups and the date of implantation of MSV (V1). Once the mobile application is made, the patients will be followed up safety and to determine efficacy as established in the visit program (V2 to V5, see later).

5. SELECTION OF PATIENTS

Patients diagnosed with low back pain and / or low back pain will be included in the clinical trial attributable to disc degeneration and evidencing the continuity of the

external fibrous ring verified by MRI. The goal is to complete 12 patients in each of the two groups.

5.1 INCLUSION AND EXCLUSION CRITERIA

5.1.1. Inclusion criteria

1. Lumbar degenerative disc disease of one or two discs with predominance of pain lumbar and / or persistent sciatica after conservative treatment (medical and physiotherapy) of more than 6 months of evolution.
2. Complete fibrous ring, capable of containing the cellular implant, demonstrated with in the MR image (Adams stages 2, 3 and 4) (Adams et al., 2000).
3. Decrease in the height of the disc space of more than 20% measured radiographically in lateral image.
4. Absence of spinal infection.
5. Hematological and biochemical analyzes without significant alterations that contraindicate intervention
6. The patient is able to understand the nature of the study.
7. Written informed consent of the patient.

5.1.2 Exclusion criteria

1. Age under 18 years old, or over 75 years old or legally dependent
2. Allergies to gentamicin, or to bovine, bovine or equine sera.
3. Congenital or acquired diseases with significant deformations of the spinal column that make application difficult.
4. Vertebral pathology that conditions segmental instability, stenosis of the medullary canal, pathology of the isthmus and other alterations that could compromise the study at the discretion of the researchers
5. Presence of Modic III changes in the RNM images (Modic & Ross, 2007) (Annex VIII).
6. Weight overload with a body mass index (BMI) greater than 35 (obesity grade II; SEEDO), where BMI = mass in Kg / (height in m) 2
7. Pregnancy or breastfeeding
8. Neoplastic disease
9. Immunosuppressive states
10. Participation in another clinical trial or treatment with another product in phase Research in the 30 days prior to inclusion in the study.
11. Any other pathology or circumstance that compromises participation in the study according to medical criteria

5.2.1 Clinical Criterion

It includes the evaluation of the state of health and quality of life through the SF-12 questionnaire (abbreviated version of the SF-36), the evaluation of low back pain (and sciatic pain if any) using visual analog scale (VAS) and disability assessment using the index of Oswestry. They are described in detail in Annexes V, VI and VII.

5.2.2 Imaging Criterion

5.2.2.1 Radiography.

The intervertebral space decrease of more than 20% in the profile projection in comparison with healthy adjacent segments is a criterion used for the purpose of inclusion or exclusion in the trial.

The classification of degeneration by discographic criteria will be carried out according to the staging Adams (Adams et al., 1986):

1. Cotton ball. No signs of degeneration
2. Lobular. Nucleus pulposus amorphous, soft and white
3. Irregular. Mature disc, with nucleus pulposus beginning to merge and with fibrous fragments
4. Cracked. Degenerated disc, with fissures and cracks in the nucleus pulposus and in the inner part of the annulus fibrosus
5. Broken. Degenerated disc, with radial fissure that reaches the outer edge of the annulus fibrosus

A lateral x-ray in standing and flexion-extension will be taken to assess the excess of mobility in the pathological segment and its evolution during treatment.

5.2.2.2 Magnetic Resonance Imaging (MRI):

T2 and STIR (short T1 inversion recovery) sequences will be performed in the axial and sagittal. They will be carried out at the beginning and 6 and 12 months after the intervention. It will be carried out quantification of the water content of the disc on the T2 image without contrast as described in detail in section 3.3 (see Figure 4). The MRI image will also be used to assess the integrity of the external fibrous ring at the inclusion visit.

5.2.3 Laboratory Analysis

They include serological determination of hepatitis B and C, HIV, lues and obtaining a sample of serum for seroteca. Although positive serology is not a criterion for exclusion of the recipient in this trial will be carried out for epidemiological purposes. The rest of the Analysis will be carried out according to usual protocols. Additional tests will be considered when clinical or complications are suspected radiographically. In the case of marrow donors

It will be necessary to carry out HIV and hepatitis serology by PCR techniques (Nucleic Acid Amplification Technology, NAT) to maximize sensitivity and thus avoid quarantine which would otherwise be necessary (Roth, 2010), but impossible to fulfill in this case. This possibility is thus contemplated in RD 1301-2006 for cells and tissues. Would convenient, and so it has been programmed, to store a tube of serum, both from the donor and of the receiver, in the Seroteca to ensure the possibility of carrying out new analyzes before situations not contemplated or not known at the present time.

5.2.4 Pathological study

It will be carried out obligatorily in case of necessary subsequent intervention on the disc.

5.3 SELECTION OF DONORS OF MESENCHYMAL CELLS

Leftover cells from patients who undergoing autologous MSV transplantation in the trial Eudra CT2009-017405-11 that we are carrying out simultaneously or in compassionate cases authorized by the AEMPS which followed the same protocol. In the above cases often we obtain more cells than needed for their own treatment in the selection process for adhesion to plastic. Informed consent will be requested (see Annex II) to donate part of the excess cells. Alternatively, if the above procedure is not possible, donors will be recruited among healthy patients undergoing spinal or hip surgery in the Service of Traumatology of the Hospital Clínico Universitario de Valladolid and who wish and consent to donate bone marrow obtained during surgery. Consent is shown reported in Annex II.

5.3.1 Inclusion criteria:

 Donors must meet all the criteria for the selection of blood donors and donation of hematopoietic precursors (RD 1088/2005, of September 16 by which establishes the technical requirements and minimum conditions of the hemo-donation and transfusion centers and services).

 They must sign the informed consent.

 They must undergo PCR serology (NAT) for HIV and hepatitis B and C and provide a serum sample for the seroteca (see details above).

5.3.2. Exclusion criteria

• Any criteria for non-compliance with the inclusion criteria

• HIV or Hepatitis positive

5.3.3 Procedure:

It is described in detail below (section 6.1). In essence, in the Eudra CT2009-017405-11 usually starts with 80-100 ml of bone marrow. The mononuclear cells and is selected by adherence to the plastic in T-175 bottles. Depending performance, which is highly variable depending on the patient, is obtained in this phase between 6 and 45x106 adherent cells, which expand about 10 times in the following 3 weeks for use in

the clinical trial. Taking into account that the required number of MSV is of about 80x106 cells, in some patients there is a significant surplus of cells in this stage. It is proposed here to start between 1 and 5 additional expansion batches (depending on the number of cells obtained) for use in the present clinical trial. The details of the protocol are discussed in section 6.1.

5.4 NUMBER OF EXPECTED PATIENTS AND RATIONALE

As it is a phase I-II study, in which the feasibility of the procedure is evaluated proposed and the safety of the product under study, the sample was fixed in 24 patients (12 for each arm of the clinical trial).

5.5 CRITERIA FOR NON-RESPONSE TO TREATMENT

It will be considered that there is no response to treatment if 12 months after the intervention there is no significant clinical changes observed in the questionnaires and / or changes in MRI images showing signs of improvement in dehydration. Shall be deemed the partial response in case of evidence of clinical or regenerative improvement in isolated.

5.6 CRITERIA FOR WITHDRAWAL OF PATIENTS AND PROCEDURES TO FOLLOW

Patients can leave the study at any time, without having to give no explanation. Additionally, patients may be withdrawn from the study for the following reasons:

1. They have adverse events that affect participation in the study
2. They do not wish to continue (withdrawal of consent) for any reason.
3. Violation of protocol or significant deviation from specific procedures of the study
4. Loss of follow-up
5. Appearance of a clinically significant intercurrent disease that affects the participation in the study.
6. The investigator considers that it is beneficial for the patient to withdraw from the study. The withdrawal of the patient from the study will not represent for him any type of damage in the control of their disease, in subsequent treatments or in the treatment to be received by the doctor. If a patient is withdrawn from the study at any time the reason must be recorded in the corresponding sheet of the data collection notebook (CRD). Patients who are withdrawn from the study due to an adverse event or abnormal results of laboratory values should be followed up to total trial resolution or for a period of time considered clinically reasonable by the researcher.

Patients withdrawn from the trial due to an adverse event will be included in security analysis. Patients who have undergone surgically intervened for graft insertion.

If a patient is lost, an attempt should be made to contact him. The investigator it should reflect the loss of follow-up in the medical history.

The aim of the study is to obtain 12 evaluable patients in each trial arm (24 in total). A patient will be considered evaluable as long as it has been infiltrated into the space intervertebral a dose of MSV or belongs to the control group. In the event that for some cause the application cannot be made to an included patient, another must be included patient in the same group until reaching the 12 patients treated in each arm of the clinical trial. The study exit and the causes of the study exit of the initial patient and no further follow-up will be necessary since they will not have received the Trial treatment.

5.8 FOLLOW-UP OF PATIENTS

The research team will inform all patients entering the study of the need to follow a specific periodic control and the importance of going to the planned visits.

To patients who experience adverse events (regardless of whether they have dropped out or withdrawn from the study) will be followed until the incidence has subsided. resolved or stabilized at a level acceptable to both the coordinator and the promoter of the study. As it is a single administration, the option of withdrawal of the medication so it is expected that these patients can continue the follow-up provided in the protocol.

All patients included in the study who received cell therapy (including patients who have voluntarily left the study or are withdrawn from it) will continue regular check-ups after participation in the study. These controls will be carried out in the Orthopedic Surgery and Traumatology Service of the Hospital Clínico Universitario de Valladolid with a frequency not less than once a year. These controls should include a clinical evaluation, an X-ray and a general analysis (acceptable if they have been carried out up to 3 months before the visit). The follow-up will be done with a minimum of 5 years. In case that any relevant information was detected for the study related to both the efficacy of the product as well as the safety of the subject (and attributable to the therapy received) is will notify the promoter, initially by telephone and, if deemed appropriate, facilitating supporting documentation (signed report or adverse event form serious) by fax or email.
In addition, and as indicated in the next point, to all patients who have received medication in the study will be given a Participation Card. At this time The research team will ask them that, in case of presenting a health problem relevant inform the doctor who attends you about your participation in said trial clinician by presenting said card. Particular attention will be paid to possibility of histological study if at any time a surgical technique is practiced on the treated area. This follow-up is not required for control patients.

5.9. STUDY PARTICIPATION CARD

All patients who have received the study medication will be given a identification tracking card so that, if necessary, you can contact the research team. This card will have a DNI size so that it can always be carried in the patient's portfolio and will contain the following text:

IDENTIFICATION CARD:

The holder of this card, Mr / Mrs: _____ is participating or you have participated in a clinical trial of cell therapy in which you received treatment with allogeneic mesenchymal cells (Protocol code: Disc_allo_MSV). Saying treatment was administered on the day: _____ and corresponds to the number of Serie_____ If you or your doctor need additional information about the study or the treatment, or report an incident, please contact:
Principal investigator: _____
Telephone contact: _____
Service: _____
Center: _____
If it is not possible to contact the Principal Investigator, please contact with the Spanish Agency for Medicines and Health Products (C / Campezo, 1, Parque Las Mercedes Business, 28022 Madrid. Tel: 91 822 6073)

5.10 TEST DURATION

The trial will last 12 months after treating all the 24 included patients (12 in each trial arm).

6. TREATMENT AND STUDY PROCEDURES

6.1 IMP AND REFERENCE THERAPY

The product or drug under study is, in the control arm, the usual treatment based on of rehabilitation physiotherapy, postural hygiene and pharmacotherapy together with the injection of 2 ml 1% mepivacaine (obtained by mixing 1 ml of 2% mepivacaine with 1 ml of serum physiological saline) in the area of the paravertebral muscles close to the affected segment, using the same maneuvers as in the intervention in the experimental arm. (see section 2.4). In the experimental arm, the investigational drug or product is MSV, which are bone marrow mesenchymal cells processed according to the IBGM system from Valladolid, suspended in isotonic Ringer-lactate medium with human albumin at 0.5% and 5 mM glucose. Cell dose: 25x106 ± 5x106 MSV, in a volume of 2 ml of cell suspension. Stability demonstrated is> 12 hours at 4-20 ºC. The whole process of obtaining MSV is carried out by highly qualified specialists in crops cell phones and is developed in the Cell Production Unit of Citospin (Parque Científico de

la UVa), meeting GMP criteria and following protocols approved by the AEMPS (PEI No. 10-134).
In the validations carried out, MSV has been shown to possess the characteristics typical MSC defined by the Society for Cellular Therapy (Dominici et al., 2006): 1) Adherence to plastic in cultivation; 2) Expression of CD73, CD90 and CD105 antigens. 3) Absence of hematopoietic antigens, markers of monocytes, macrophages and B lymphocytes.
4) In vitro differentiation capacity in osteoblasts, adipocytes and chondrocytes. Watch details in Figures 2 and 3.

6.1.1. Obtaining, cultivating and expanding MSV.

The processing and multiplication of harvested bone marrow cells using the IBGM procedure will last 3-4 weeks. The responsible medical team is expert in the management of the selection, culture and collection procedure of the cell product that, in its status available for application, is called MSV (the V added to the initials usual ones comes from Valladolid, to indicate that it follows the procedure developed, validated and authorized in our Cell Production Unit)).
As a cellular source for obtaining MSV, bone marrow aspirate is used from healthy donors submitted to the protocolized anticoagulant procedure. The protocol of obtaining is described in sections 5.3 and 6.1.6 and the consent documentation reported is included in Annex II. Once the bone marrow sample has been received (in a period less than 12 hours) and after checking the perfect condition of the shipment and the correct Completion of the accompanying documentation (informed consent of the donation, serologies, NAT etc), the bone marrow processing will be carried out in the Citospin facilities, in the Science Park of the University of Valladolid. In essence, the mononuclear cells are separated from the bone marrow aspirate by density gradient with Ficoll and subsequently a feasibility study is carried out mobile. During cultivation * 2 trypsinization processes or passes will be carried out, which have as an objective multiplying and purifying the cell line. In order to ensure that cells in culture are really MSV and the achievement of the established dose, the culture of will last for 3-4 weeks, until you have a sufficient number of cells (25x106 cells / intervened segment). The details of the specifications are those that appear in Production Guide (Preparation of Cell Therapy: expansion of mesenchymal cells bone marrow: FA-CMES-001-P). Serum samples will also be obtained from donor to carry out the mandatory serologies (RD 1301-2006, for cells and tissues human), performed using the PCR technique (NAT by Nucleic Acid Technology) to avoiding quarantine (Roth, 2010) and keeping a sufficient sample in the seroteca to further analysis, if necessary.
It has recently been conclusively shown that, contrary to what is stated in previous publications, there is no malignancy or genetic alterations in SCD maintained and expanded in vitro for prolonged periods (Bernardo et al., 2007; Bernardo et al., 2009; Bernardo et al., 2011), and that the initial errors were due to contamination with other immortalized cell lines grown in the same laboratory (de la Fuente et al., 2010; Torsvik et al., 2010; Vogel, 2010). At the end of the process, the characterization of the MSV will be carried out through studies immunophenotypic using flow cytometry, using the markers established for this type of cells, a higher positive cell phenotype should result (> 95%) for CD73, CD105, CD90, CD166 and negative (<10%) for CD45, CD14, CD34, CD31 and HLA-DR.
The cells will be packed in 5 ml syringes suspended in 2 ml of isotonic solution. Ringer-lactate, supplemented with human albumin (0.5%) and glucose (5mM). The number of cells administered will be 25 ± 5x106 cells. The sterility, cleanliness and quality control conditions of the production unit will be maintained following the steps defined in the Standard Procedures for Work established by the GMP norms of the "Cell Therapy Unit of the IBGM".

6.1.2. Conditioning and transport for shipment.

Once the MSVs are obtained, they will be resuspended in 2 ml of Ringer-lactate isotonic medium, supplemented with human albumin (0.5%) and glucose (5mM) to be

loaded into a 5 ml labeled syringe. This product has a stability of more than 12 hours at room temperature according to our protocol. The medicine will be transported in refrigerators validated for such activity, keeping it between 4 and 16 oC.
It has been found that, in these conditions, it fully retains viability for> 12 hours (Orozco et al.,
2011).

6.1.3. Cell application.

Antibiotic prophylaxis will be performed with 2 g of IV cefazolin or 1 g of IV vancomycin in case allergy. The implantation of the cells is carried out under light sedation and radiological control. The cells are injected by puncture of the disc avoiding the neurovascular elements (Konings & Veldhuizen, 1988). After double brushing the region with a aqueous solution of povidone iodine (or chlorhexidine in patients allergic to iodine), The field is delimited with sterile sheets and local anesthesia (Scandicain 1%) is applied to the skin, subcutaneous tissue and muscle near the puncture. With fluoroscopy in position anteroposterior, a vertical line is marked on the skin corresponding to the projection of the spinous process with a sterile dermographic pencil. Then a line is drawn perpendicular corresponding to the projected image of an aligned Kirschner wire with the intervertebral space to be treated and a 20G spinal needle is inserted with an inclination 25 to 35 degrees toward the midline at a point 8 to 9 cm (depending on the patient morphotype) from the midline. At this time, fluoroscopy is switched to lateral position to ensure needle penetration follows the correct direction until the nucleus pulposus is reached. After verifying the correct position of the needle in the nucleus pulposus both in the anteroposterior fluoroscopic view and in the side, the cell suspension is slowly injected. Using this procedure, do not we had incidents in none of the 10 patients in our previous study (Orozco et al., 2011).

6.1.4 Post-treatment regime

The patient is discharged after a 2-hour observation period. Not prescribed Lumbostat corset and walking is allowed for moderate periods. Activity is suspended work for a week. Exercises to tone the muscles are prescribed para-vertebral and abdominal, which begin one month after the intervention. Medication analgesic is adapted to the needs of each patient and use should be avoided as far as possible of anti-inflammatories. Long-term follow-up will consist of clinical visits and monitoring Radiographic and MRI as specified in point 7.2 (“Schedule”).

6.1.5. Possible complications derived from the proposed therapy.

The complications are the same as in the discography, and they occur very rarely. The most common complaint is exacerbation of pain for 1-2 weeks, which usually resolved by analgesia and muscle relaxants for a period short. The most serious complication is discitis, the incidence of which is less than 0.1% (Osti et al., 1990). Other complications described, although much less frequently, are transient headache, nausea, meningitis, epidural abscess, arachnoiditis, bruising intrathecal, retroperitoneal hematoma, cauda equina syndrome and acute disc herniation. Isolated cases of urticaria are not considered in this trial, as they are attributable to contrast medium. Initial fears about possible side effects of the Long-term discography regarding disc viability have not been confirmed. In a study with up to 20 years of clinical follow-up, no radiographic evidence was found of progressive disc degeneration after discography (Flanagan & Chung, 1986). The possible risk of complications derived from the use of allogeneic cells has been discussed in detail above. Suffice now to summarize that, according to the literature, the application of Allogeneic MSCs, both locally and systemically, have been shown to be safe both in the context of clinical trials and studies in animal models. Either way, will monitor skin and mucosal reactivity to watch for a possible immune reaction.

6.1.6. Methodology for obtaining bone marrow from donors.

This procedure will be applied to donors, generally included in the Eudra trial. CT2009-017405-11 (MSV_Artro) that we are carrying out or in compassionate cases authorized

by the AEMPS, and that follow the same protocol and that provide their cells left over for this test (see section 5.3).
The methodological protocol will be the one described in MSV_Arto: Marrow puncture-aspiration bone will be performed in the outpatient surgery operating room in a scheduled session. The patient in the prone position, accommodated with pillows, will undergo mild sedation. Then the surgical field will be brushed with alcoholic povidone-iodine solution (chlorhexidine if there is a history of allergy to iodine) and delimitation with sterile fields leaving both posterior iliac crests free. After local anesthesia (10 cc + 10 cc of 1% lidocaine, without vasoconstrictor) and sedation, two members of the extraction team, located at both sides of the operating table, with a 11 G trocar, various punctures located under the posterior superior iliac spine towards the joint sacroiliac (this is the area of the iliac with the highest trabecular density). The technique involves the Cortical perforation and sudden aspiration of a maximum of 1mL of bone marrow to avoid in peripheral blood aspiration is possible. The material obtained is injected into the bag supplied for transport that has previously been heparinized with an adequate dose to blood volume. The 2 successive aspirations are carried out by rotating 90º in the direction of the beveled trocar clockwise. The same puncture hole allows 1-2 depth mm about two times, repeating the same 1 mL aspiration methodology, change of syringe, 90º bevel rotation and new aspiration. The trocar can then be removed slightly and by sliding it a few millimeters over the cortex of the pelvis, the puncture, continuing on both sides of the pelvis until about 80 mL is achieved. immediately transport the marrow (sterile heparinized bag with a medium volume of 80 mL of aspirated bone marrow) to the Citospín Cell Therapy Unit, Parque UV scientist, under regulated conditions and room temperature (15 to 25 ºC) in a maximum of 12 h.

6.2. TREATMENT ASSIGNMENT

6.2.1. Numerical correspondence of patients

The patients will be identified with a correlative number until the inclusion of the 24 patients (example: 001, 002, etc). In the event that an initially enrolled patient is need to be replaced, the assigned number would be the same one preceded by an R (example: R01, R02, etc).

6.2.2. Randomization numbers

The study population will be made up of 24 patients with low back pain attributable to degenerative disc disease that is refractory to conventional treatment and with integrity of the external annulus fibrosus demonstrated by MR imaging. Of this cohort, 12 patients will be included in the experimental group and another 12 in the control group. Will be assigned to one or the other randomly. It will be carried out by the CRO centrally and at random, attending to the treatment to be administered to each patient (control or experimental). The assignment will be kept in a sealed and identified envelope.

6.3. CONCOMITANT TREATMENTS

Relevant concomitant medications taken by the patient will be recorded in the CRD regularly due to other parallel treatments allowed during the study. If analgesic treatment is necessary, it will preferably be carried out with paracetamol. I know avoid administering corticosteroids or anti-inflammatory therapy if they are not strictly necessary. If necessary, it should be noted in the clinical history and in the CRD (Section "Concomitant Medication").

6.4. ACCOUNTING OF THE DRUG

The product under study or medicine is produced individually by starting from the bone marrow from each donor. In all cases, the MSV will be registered and identified. produced for each patient in the IBGM.

6.5. MANAGEMENT OF STUDY MEDICATION

The study medication, properly packaged MSV in a thermostatic container, was transported immediately after production to the application center. Has a stability longer than 12 hours at room temperature and is given as an injection in the area of the nucleus pulposus of the affected spinal segment.

7. DEVELOPMENT OF THE TEST AND ASSESSMENT OF THE RESPONSE

7.1 ASSESSMENT OF THE RESPONSE

7.1.1. Main evaluation variable

The objective is the evaluation of the efficacy using clinical criteria (Visual evaluation back pain analogue-VAS-, Oswestry disability index -ODI-, quality of life --SF-12--), and imaging (quantitative MRI) at 6 and 12 months after intervention.

7.1.2. Secondary evaluation variable

There are no secondary variables for efficacy.

7.2 TEST SCHEDULE

VER TABLA EN LA PAGINA SIGUIENTE

CRONOGRAMA	**Preinclusión**	**Inclusión**	**Tratamiento**	**Seguimiento (m=meses)**			
PROGRAMA DE VISITAS:		**V0**	**Día 1 V1**	**Día 8 V2**	**3 m V3**	**6 m V4**	**12 m V5**
Historia Clínica y Exploración Física	**X**	**X**		**X**	**X**	**X**	**X**
Entrega de la Hoja de Información	**X**						
RX Columna Lumbar Simple y dinámica	**X**					**X**	**X**
Firma del Consentimiento Informado		**X**					
Serologías víricas preceptivas		**X**					
Comprobación de criterios de Inclusión y Exclusión		**X**					
RM lateral, T2, cuantitativa		**X**				**X**	**X**
EVA, Oswestry, SF-12		**X**			**X**	**X**	**X**
Tratamiento control o con MSV alogénicas*			****X**				
Analítica Control		**X**		**X**		**X**	**X**
VISITA:		**0**	**V1**	**V2**	**V3**	**V4 M**	**V5 12 M**

* Las MSV deben haberse obtenido del donante y expandido en la ventana temporal adecuada. **RX en quirófano para guiar y seguir aplicación. La RM de Vo se usará también
para valorar la integridad del anillo fibroso externo.

7.2.1. Visiting Program

Pre-Inclusion: In patients who come for consultation and whose medical history and examinations complementary, including X-rays and / or MRI, presume an orientation diagnosis with the possibility of inclusion in the trial, presenting all the criteria of inclusion and none of exclusion, the treatment regimen will be prescribed according to the standard and then they will be informed of the possibility of inclusion in the EC. They will deliver the "Patient Information Sheet" (Annex II) and, if its decision is affirmative, Inclusion procedures will begin on a future visit.

Visit 0. Inclusion. May comprise more than one physical visit. If the patient confirms that want to join the trial, you will be asked to sign the “Consent Document Informed ”(Annex II). You will be ordered new MRI and radiographic scans with the mandatory characteristics of the study unless these examinations had already been performed previously within a period of no more than 6 months. Completed analytics will be requested with the regulated serology for determination of HIV, Hepatitis and Lúes and test of pregnancy if you are a woman of childbearing age. Although positive serology is not a reason for exclusion in this trial, it will be done for epidemiological reasons. After checking the inclusion and exclusion criteria, the clinical history will be made, including data referring to low back pain and discopathy, treatments carried out, analytics and imaging tests to define the characteristics of the same and carry out the measurements that will be taken as a standard of future explorations. Low back pain evaluation questionnaires will be passed (VAS), disability (Oswestry) and quality of life (SF-12). It will be scheduled on the day of the intervention (V1) and the X-ray and MRI tests will be performed, if they had not already been performed.

Visit 1 (day 1). Placebo injection or allogeneic MSV, depending on the clinical trial arm.

Visit 2 (8 days): Safety assessment. Includes analytical control.

Visit 3 (3 months): Safety assessment. EVA, Oswestry and SF-12 questionnaires. RX.

Visit 4 (6 months): Safety assessment. EVA, Oswestry and SF-12 questionnaires. RX. RM.

Visit 5 (12 months) Safety assessment. EVA, Oswestry and SF-12 questionnaires. RX. RM.

8. ADVERSE EVENTS (AA)

8.1. Definition

Adverse event is any unfavorable medical event that occurs in a patient or subject undergoing clinical investigation who is administered a product Pharmaceutical, regardless of whether or not it is considered related to the product pharmacist. Any sign (including alteration of a laboratory parameter), symptom or disease, unfavorable and unintended, that appears associated temporarily with the use of a medicinal product can constitute an event adverse.

Unexpected adverse events are those experiences not described (in nature, severity or frequency) in the technical data sheet of the product.

8.2 Description of adverse events

Previous publications have shown that the interventions proposed in this rehearsal are safe. Our preliminary clinical experiences with autologous MSV and in General MSC have not identified any Adverse Health or Safety Event caused or associated with them.

Possible risks related to sedation combined with necessary local anesthesia for bone marrow aspiration in the MSV donor group are: hypoxemia, arrest respiratory, aspiration pneumonia, hypotension and death. However the probability of a complication of this severity should be considered exceptional.

Possible risks related to donor bone marrow aspiration are:

 Minimal bleeding from a puncture site; minor complication, only requires a compression bandage.

 Feeling of nausea and instability in the hours following sedation. By This reason is usually kept in hospital for the next 2 hours to obtaining.

 An allergic reaction to any of the anesthetic drugs used is considered an exceptional complication, with an incidence of less than 1 in 50,000 anesthesia.
 Fever, sometimes a few tenths in the first hours after obtaining.
 Pain in the puncture sites that is quickly controlled with pain relievers common, paracetamol type, and usually disappears in less than 48 hours. To promote its resolution, it is recommended to take a few days of relative rest.
Interventions carried out during treatment (local injection of mepivacaine or Allogeneic MSV) derived from product infiltration are minor, and attributable to the diagnostic recording technique, namely, the risk associated with the sedation, possibility of infection or bruising, death. However, it is understood that the treatment proposed in this study does not imply risks greater than those of surgery habitual of the contemplated pathology. Rather, it involves avoiding a technique open surgical procedure and the implantation technique is the same as that used in the discography, a widely used and widely used diagnostic technique experience. Sedation and local anesthesia are assumed to maintain very high risk levels. low under conditions of good clinical practice Aside from local pain or swelling, the arm of allogeneic MSV may have theoretically immunological rejection, although, as detailed in another section, MSCs have little or no antigenic power and this type of phenomenon has not been described or in animal experiments (rabbit and pig) or in clinical trials conducted in patients with lupus erythematosus ((Sun et al., 2009; Liang et al., 2010).have obtained similar results with allogeneic mesenchymal cells derived from cord blood (Sun et al., 2010) There are also numerous treated cases of patients with breast cancer (Koc et al., 2000) graft versus host disease (Lazarus et al., 2005), and other autoimmune diseases (Bernardo et al., 2009; Bernardo et al., 2011) without that there have been, so far, adverse effects. Finally, there are 44 declared trials clinicians using allogeneic MSCs in the “clinicaltrials.gov” database for treatment of very varied pathologies, including not only graft-versus-host disease and different autoimmune diseases, but also ischemic strokes, cardiac or peripheral disorders, liver destruction, osteogenesis imperfecta or degeneration of the intervertebral disc (see http://clinicaltrials.gov/ct2/results?term=mesenchymal+allogenic). On summary, both animal experimentation and use in human clinical trials demonstrates the safety of allogeneic mesenchymal cells, without having reported adverse effects. The collection of Adverse Events must be carried out by the researcher responsible for the trial, describing it according to its duration, intensity, frequency and relationship of causality between the treatment and the adverse event. The researcher should ask indirect questions directed to the patient at each visit of the study. Adverse events can also be reported voluntarily by the patient during or between visits or detected in physical examinations, laboratory tests or in other evaluations. In the Data Collection Notebook (CRD) of the study, the researcher must record in reference to A: A. Duration: refers to the time elapsed since the occurrence of the event until completion.
B. Intensity: it will be defined according to a scale of three gradations:
 mild: does not interfere with the subject's daily life
 moderate: interferes but does not impede normal activity.
 severe: interferes with some important aspect of daily life and can even force to abandon the treatment.
C. The frequency: it will be defined as: punctual, recurring or persistent.
D. The causal relationship: it will be established according to the classification in which the following categories:
 Not related
 Unlikely
 Possible
 Probable
 True

8.3 Record of Adverse Events

Adverse events observed during the clinical trial, either either by the researcher or by the patient himself, they must be recorded in the description of adverse events. This form can be found in the CRD of each participant in the study.
Adverse Events will be recorded from the moment the patient signs informed consent. All of them will be logged, regardless of the relationship of imputed causality.

8.4 Serious Adverse Event (AAG)

8.1.4.1 Definition

A Serious Adverse Event is any unfavorable medical event that any dosage of the product or due to specific study procedures:

- Produces the death of the patient,
- Threatens life,
- Produces permanent or significant disability,
- It gives rise to hospitalization or prolongation of it, (hospitalizations scheduled prior to patient selection or study procedures, will not be considered AAG).
- Congenital anomalies and malignant processes.

8.1.4.2 Notification of Serious Adverse Events (AAG)

The investigator is responsible for the immediate reporting of adverse events. serious or unexpected. The investigator must notify any AAG to the CRO (FTH) in the within one working day from the moment the investigator learns of the existence of the serious or unexpected adverse event, regardless of the causal relationship.
The minimum information that an initial report of a serious adverse event must collect (It can be notified by phone, fax or via email) is:

- name of the researcher,
- protocol code,
- study drug,
- patient identification number,
- description of the event,
- start date,
- severity criteria and
- causality of the event

The investigator should send the trial monitor within 24 hours of the When you become aware of an AAG, the Notification of AAGs form (Annex IV) completed, signed, dated and with all the information available at the time.
In the event that the AAG or unexpected has caused the death of the subject or placed in
danger to his life, the investigator will contact the trial monitor and send the form Serious Adverse Event Notification Form completed and signed and dated on the within 24 hours from the moment the researcher becomes aware of the same.
The CRO (FTH) will act on behalf of the Promoter to notify the AEMPS, the CEIC that is applicable and the competent bodies of the applicable Autonomous Communities, the suspicions of serious and unexpected adverse reactions that may be related to the investigational drug. Likewise, all the participating researchers.
Unexpected causal related AAGs will not be notified to the competent authorities with other procedures of routine clinical practice in the treatment of these pathologies, particularly those of surgical procedures. Expected serious adverse events, non-serious adverse events and those that are considered unrelated to the treatments under study will be included in a tabulated in the annual or final report of the clinical trial.

9. ETHICAL ASPECTS

9.1 ETHICAL AND REGULATORY REQUIREMENTS

The study will be carried out in accordance with the protocol, the ethical principles based on the latest version of the Declaration of Helsinki (WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI. (Ethical Principles for Medical Research Involving Human Subjects) (Annex III), in the Guidelines of the International Conference on Harmonization (ICH) on Good Clinical Practice (ICH Topic E: Good

Clinical Practice. Note for Guidance on Good Clinical Practice. CPMP / ICH / 135/95 and to any new version or modification existing; as well as applicable national and / or international legislation.

9.1.1 Investigator Responsibilities

Before starting the study, the Investigator must sign a protocol signature page confirming that you agree to carry out the study in accordance with these documents and with all the instructions and procedures described in the protocol and that, when required, will allow designated monitors, representatives of the Warranty Department of Clinical Quality (CQA) and the Health Authorities have access to all the data and relevant records.

The Investigator is responsible for providing patients and / or their representatives with adequate oral and written information about the study. All questions about the study must be answered to the patient and / or their legal representatives.

9.1.2 Informed Consent

Eligible patients can only be included in the study after providing for written informed consent previously approved by the CEIC (Annex II), or, in the case of inability to do so, after said consent has been granted by a legal representative of the patient, or in the event that he lacks a legal representative the person linked to the patient for family reasons. Informed consent must be signed and dated by the patient and the investigator, who will have provided each patient (or a representative of the patient) prior to inclusion in the study, information complete and adequate verbal and written study objectives and procedures, as well as as the possible risks that it may entail. The bone marrow donor will thus receive himself, an information sheet and will sign a consent authorizing the use of his cells for allogeneic transplantation (see Annex II). Informed consent must be obtained before starting any procedure. study specific (i.e. all procedures described in the protocol). The process of obtaining informed consent must be recorded in the documents patient source.

The proposed informed consent forms are provided in ANNEX II. that meets regulatory requirements and is deemed appropriate for this study. The informed consent / patient information sheet includes the items listed in the ICH Guideline on Good Clinical Practice. Any change in the Informed consent proposed by the investigator must be approved by the Promoter and notified to the corresponding CEIC.

9.1.3 Approval of the Clinical Research Ethics Committee (CEIC)

Before starting the study, the written, signed and dated approval of the protocol, informed consent and patient information sheet by the CEIC applicable according to current national legislation. The approval must be saved in the file of the researcher in the center and a copy in the file of the Promoter.

9.1.4 Approval of the Health Authorities

Before starting the study, all established regulatory requirements must be met and the clinical trial must be approved. The Promoter or representative of the Promoter together with the Researcher will request the approval of the Health Authorities. This approval is will be kept in the Researcher's file at the study center, with a copy in the file of the Promoter.

9.2 MODIFICATIONS OF THE PROTOCOL

Any changes or additions to this protocol can only be made through a Written amendment to the protocol, which must be approved by the AEMPS and CEIC corresponding, when required according to current applicable legislation. All amendments to the study protocol will be made in writing and filed in the corresponding study file, as a modification of the protocol. Are Modifications must be documented, dated and signed by the investigator and the Promoter.

9.3 INSURANCE POLICY

The promoter of the study, will arrange an insurance that covers the damages and losses that as a consequence of the same could result for the person in which it is to be carried out: Said civil liability insurance will cover the responsibilities of the Promoter, the researcher and his collaborators and the head of the hospital or center

where the trial is carried out and will comply with current applicable legislation. Policy is attached.

9.4 ACCESS TO PATIENT DATA

The patient's medical information obtained by conducting this study is considered confidential and its disclosure to third parties is prohibited. The confidentiality of the patient is insured since they will be identified only by their initials and a code number of the assigned study.

The investigator will inform patients that their identity will be strictly maintained confidential. During the study, the patient will be guaranteed strict compliance with the Law 15/1999 of December 13, Protection of Personal Data. The researcher, when processing and treating the patient's data, will take the necessary measures appropriate to protect them and prevent access to them by unauthorized third parties. The patient may revoke the informed consent for the processing of their data personal, addressing the investigator.

The data generated as a result of the clinical trial must be available to be inspected at the request of the Promoter, the CEIC or the Health Authorities. The signing of the informed consent means that the patients explicitly accept that the study data may be consulted by the researcher and his team, the representatives of the Promoter and if required by the CEIC and Health Authorities.

10. PRACTICAL CONSIDERATIONS

10.1 RESPONSIBILITIES OF THE VARIOUS PARTICIPANTS IN THE TRIAL

10.1.1 Investigator

The researcher must comply with all the requirements of the protocol, he will be responsible for the information is accurate and truthful and has been obtained in the manner indicated therein. In particular, the investigator must ensure the best possible care at all times. of the patient always putting their safety and well-being first.

10.1.2 Monitor

The monitor must confirm that the information collected in the CRD is true, for which must have the collaboration of the research team. In addition, you will be responsible for communicate to the Sponsor any serious or unexpected adverse events that occur during the study.

10.1.3 Promoter

The Promoter will be responsible for ensuring compliance with the ICH Directive on Good Clinical Practice and applicable national and / or international regulations.

10.2 DATA REVIEW

10.2.1 Center monitoring

Before starting the study, a meeting of the monitor will be held with all the investigators and the protocol and the correct composition of the CRDs will be reviewed. During the study, the monitor designated for the test will regularly visit the entrance to check that patient documents are complete, data accuracy of the CRDs, the monitoring of the Standards of Good Clinical Practice (GCP) and the progress of recruitment. Investigators and all personnel involved in the trial should be available to assist the monitor during these visits.

The researcher must make available to the monitor the source documents of each study patient that will consist of the history and notes of the visit (medical records, clinical or hospital) including medical and demographic information, data from laboratory and the results of any other tests or evaluations. All the information that is registered in the CRDs, it must be verifiable with the source documents that they are usually found in the patient's medical record. The investigator must keep a copy of the signed informed consent form.

The researcher must agree to the monitor reviewing all source documents for confirm that the data is consistent with the data in the CRDs. A complete verification of the existence and correct obtaining of informed consent, of the compliance with the inclusion / exclusion criteria, the documentation of the AAGs and the record of the data

used for all efficacy and safety variables. I know carry out additional checks of the consistency of the original data with
those of the CRDs, according to the study's specific monitoring plan.

10.3 DATA COLLECTION

10.3.1 Data collection notebook (CRD)

All the data generated in the clinical part of this study will be transcribed in the CRDs they will be printed in triplicate (carbonless paper). All data will be transcribed and modifications will be made in black ink. Any correction of the data must be signed, dated and with the reason for the modification noted. Only authorized personnel can enter data and make corrections in the CRD. The Investigator and study monitor will review all CRDs to ensure they are complete and legible and accurate. The monitor will instruct the center staff to make any necessary corrections or additions. The study monitor will have access to the medical records, original laboratory data and other related documents with the study, without compromising the identity of the patients. CRDs should be completed on a regular basis to keep data up to date and reflect the patient's condition in each period of the trial. The information collected and entered in the CRD will correspond to that of the source documents.

Patients will not be identified by name on the CRD, an ID will be used coded consisting of a patient number and initials. The researcher must carry out a confidential record (patient identification list) containing information about identification of the patient included in the trial in order to keep track of the themselves if necessary.

10.3.2 Original data

The original data are those that provide evidence of the existence of the patient and they ensure the integrity of the data collected. All data on the patient's participation in the study and clinical status during the period of the study should be noted / filed in the patient's medical record. This data is defined as original data. Medical information relevant to the efficacy and safety evaluation will be transcribed to specifically designed CRDs for the study and also collected in the clinical history. During monitoring visits, consistency between stories will be checked patient clinics and CRDs, that is, verification of original data (SDV).

These, in case there is any discrepancy, must be justified.

10.4 DATA CONSERVATION AND RECORD KEEPING

All data related to the study, including the contents of the file of the study, CRDs and original data will be stored by the researcher for a period at least 15 years old. The investigator will keep a copy of each CRD and the original will be kept will send to the Promoter.

The investigator's file will contain the documents described in the ICH Directive on Good Clinical Practices.

The clinical records of the patients and other original data will be kept as much as possible period of time allowed by the hospital, institution or private practice where the performed the test. The Promoter will keep all the remaining documentation related to the trial during the period validity of the drug.

10.5 QUALITY CONTROL

During the development of the trial, monitoring visits will be carried out periodically to the centers to ensure that the protocol requirements are followed.

10.6 INSPECTIONS AND AUDITS

The Promoter, either directly or through contracted third parties, may carry out quality assurance audits at participating centers. In the event of audits, the auditor will have access to medical records, files and relevant documentation related to the clinical trial. Health Authorities may carry out inspections during the study and after its ending. In the case of audits and / or inspections, the researcher will provide all the documentation related to the study.

10.7 PRESENTATION AND PUBLICATION

All information regarding this clinical trial is considered strictly confidential and is the property of the Sponsor, who may use the Study data for scientific or commercial. Any

information related to the study will not be communicated to third parties without the prior written authorization of the Promoter.
The publication or presentation, whether complete or partial, of any part of the data or Trial results will be subject to review and written agreement with the Promoter.

11. STATISTICAL ANALYSIS

11.1 SAMPLE SIZE

As it is a phase I-II study, in which the safety of the product under study is evaluated, the sample was fixed at 12 patients per branch (24 patients in total)

11.2 STATISTICAL METHOD USED

In all descriptive analyzes, categorical variables will be summarized using frequencies and category percentages. For the distributions of continuous variables, will present the mean and its Standard Deviation, the median and the 25th and 75th percentiles of the distribution, the number of cases, minimum and maximum value.
All hypothesis tests will be two-tailed, with a significance level of 5%. The The result of a hypothesis test will comprise the 95% Confidence Interval of the variable (s) analyzed. To calculate the statistical significance of the differences between the two groups, a normality analysis of the distribution will be carried out and compare the means using the double-tailed t-test or the Wilcoxon, depending on whether the population is Gaussian or not.

11.2.1 Main objectives, main variables

- Evaluate the feasibility and safety of the use of allogeneic MSV injected into the disc space for the treatment of lumbar degenerative disc disease and pain and the disability it causes.

The variables that will be used to evaluate safety will be the AAG number related to study medication and the percentage of patients who presented a related AGS.
-Evaluate the analgesic-anti-inflammatory efficacy determined by the variation of the Low back pain assessment questionnaire scores (VAS), disability (Oswestry) and health and quality of life (SF12) at 3, 6 and 12 months.
- Evaluate regenerative efficacy using quantitative MRI imaging criteria determined by the appearance of improvements in disc dehydration at 6 and 12 months.

11.2.1.1 Radiographic criteria

Adams stage 2-4 classification (Adams et al., 1986), with (fibrous ring capable of contain the cell implant), used for inclusion or exclusion purposes in the assay.
In profile radiography carried out with the same technical characteristics and times (before noon) the height of the intervertebral space in millimeters will be measured, this being the
variable that will be compared with the measurements that occurred over time (6 and 12 months after MSV implantation). An increase in height will be understood as an indication of rehydration disc associated with an increase in proteoglycans and cellularity of the NP. Descriptive analysis of the observed values will be expressed by the mean, standard derivation, interval of 95% confidence of the mean. To find out if there are differences between baseline and at 6 and 12 months the Wilcoxon nonparametric test for paired data will be used. So Optional, the study will be repeated at 12 months evaluating changes in the leading. The analysis descriptive of the observed values will be expressed by the mean, standard deviation, 95% confidence interval of the mean.

11.2.1.2 Quantitative MRI criteria

Quantitative density evaluations will be carried out in the “ROIs” (Regions of interest) of the area of the nucleus pulposus of the affected segment and of the 3-5 healthy neighboring segments in the T2 images taken before the start of treatment and 6 and 12 months after inclusion. The quotient of the value obtained in the affected segment divided by the mean of the healthy segments will be used as a measure of the relative water content, with 1.00 being the normal value. The same ROIs should be used for the measurements obtained at 3 times, before treatment and 6 and 12 months after. The procedure is illustrated in Figure 4 (taken from (Orozco et al., 2011)). The increase

in the quotient approaching the value of 1 is will consider a sign of rehydration. The descriptive analysis of the values of this variable It will be expressed by the mean, standard deviation, 95% confidence interval of the mean.
To find out if there are differences between baseline and at 6 and 12 months, the no test will be used. parametric for paired Wilcoxon data.

11.3 POPULATION AND ANALYSIS

Assessment definitions that are created to define the populations of the analyzes in this studio.

11.3.1 Evaluable patients

A patient will be considered evaluable provided that he has undergone the specified interventions (Control or allogeneic MSV).

11.3.2 Efficacy population (Per protocol (PP) population):

Secondary efficacy endpoint analysis will be based on all patients who do not have incurred in major protocol violations, and have at least one rating final efficacy of the graft.

11.3.3 Safety population:

It is the population that includes all evaluable patients who have undergone one of the three study treatments. The security population will be the population of analysis of the safety variables of the treatment.

11.3.4 Analysis methods

Descriptive statistics will be made including summary statistics and lists. For the comparisons of means at 0, 6 and 12 months a comparison of means using the t-test or Wilcoxon test as appropriate (see 11.2).

11.3.5 Background and demographic characteristics

Lists by patient of all demographic measures and baseline data will be presented, tabulating the following numerical variables: number of valid cases, number of values not available, mean, standard deviation, 95% confidence interval of the mean, median, minimum and maximum. For the discrete and categorical variables, will present the frequency and percentage of each value.

11.3.6 Security

 AA and AAG lists will be made showing relationship, causality and severity.
 Frequency and percentage of AAG
 Frequency and percentage of AA
 Laboratory parameters including hematology and biochemistry: leukocytes, absolute neutrophil count, platelet count, creatinine, AST (SGOT) / ALT (SGTP), etc. Laboratory parameters will be listed by patient and visit and include the reference range values. These parameters are will be summarized by baseline and post-baseline visit analysis. The analysis will include "n" valid, mean, standard deviation, median, minimum and maximum. In addition, the change from baseline will be evaluated as described in 11.2. All the Statistical analyzes will be performed with a bilateral significance level of 0.05.

11.3.7 Efficacy

Changes in pain, disability and quality of life indices will be described in the baseline situation and at 3, 6 and 12 months of treatment, which will be compared as specified in 11.2.
The frequency and percentage of patients presenting signs of regeneration at 12 months of the different interventions, comparing the values as specified in 11.2.

11.4 SOFTWARE

The statistical analysis will be carried out using the statistical package InStat (v.3.06. GraphPad
Software, Inc).

11.5. DIFFICULTIES AND LIMITATIONS OF THE STUDY

As noted above, previous clinical trials show that a considerable improvement (up to 30%) due to the placebo effect. It will therefore be accurate discount this random effect before assessing the effects of MSVs. It is therefore, important evolution in the control group.

Bone marrow donation could be a problem if not enough were found donors. The solution to be used, when possible, by patients undergoing treatment with autologous mesenchymals (in the trial we are conducting in collaboration with CM Teknon) also as allogeneic donors would be the situation ideal, the most economical in terms of required means and the most interesting scientifically.
We have tried to obtain the maximum masking in the test within a design reasonable for this first study. While the “single blind” (patient) design is feasible without serious complications, the “double blind” (researcher) or “triple” (analysis) various logistical and ethical issues, so they have been left for the future. If there are indications of efficacy for allogeneic MSV, it will not be possible to perform direct comparison with autologous MSVs, so if these are obtained results, a new trial should be designed to directly compare the efficacy of both therapeutic alternatives.

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ANNEX I: PRODUCT DOSSIER / INVESTIGATOR'S MANUAL

The product under study is made up of allogeneic mesenchymal cells (MSV) isolated and expanded under GMP conditions following our protocol authorized by the AEMPS (PEI Num. 10-134; a copy of the notification is added at the end of this document). The details of this product are briefly described below and with more detail in section 6.1 of the Protocol report.

General characteristics of the product under study.

The investigational drug or product is allogeneic mesenchymal cells from bone marrow, and processed according to the Standard Work Procedure of the IBGM of Valladolid (MSV), suspended in isotonic Ringer-lactate medium with albumin 0.5% human and 5 mM glucose. Cell dose: 25x106 ± 5x106 MSV, packed in 5 ml syringe containing 2 ml of cell suspension and labeled with the name "Disc_MSV_allo" and the reference numb er. Demonstrated stability is> 12 hours at 4-20 ºC (Orozco et al., 2011). The entire process of obtaining the MSV is carried out by qualified specialists in cell cultures and NCF, and is developed in the Unit of Cell Production of the IBGM (UVa Scientific Park), meeting GMP criteria and following protocols approved by the AEMPS (PEI No. 10-134). The procedure of collection, cultivation and expansion is detailed in section 6.1.1 of the protocol. In the validations carried out, it has been shown that the MSV have the typical characteristics MSC defined by the Society for Cellular Therapy (Dominici et al., 2006):

1) Adherence to plastic in cultivation;
2) Expression of CD73, CD90 and CD105 antigens.
3) Absence of hematopoietic antigens, markers of monocytes, macrophages and B lymphocytes.
4) In vitro differentiation capacity in osteoblasts, adipocytes and chondrocytes. The cell product, **MSV**, is the same that we used in the EudraCT 2008-001191- assay. 68; intervertebral disc) whose results demonstrate feasibility and safety and have been recently published (Orozco et al., 2011). We are using the same product in the Eudra-CT 2009-017405-11 (NCT01183728, autologous MSVs in knee osteoarthritis) and Eudra-CT 2011-005321-51 (NCT01586312; allogeneic MSV in gonarthrosis) with hopeful indications of efficacy and without there having been, for the time being, adverse effects.

Our Cell Production Unit of the IBGM, according to GMPs, directed by Dr. Ana Sánchez, was the first Clean Room authorized by the AEMPS in the Public System and currently participates in eight clinical trials, several of them with mesenchymal cells of the bone marrow. It has an MSC preparation protocol authorized by the AEMPS (PEI No. 10-134, Valladolid mesenchymal cells or MSV). It has a stable staff with training in quality control, which includes 12 people. Had given recently originated a Technology-Based Company owned by the University, Citospin S.L. that adds additional nuances to cell production and features new and modern facilities in the Science Park of the University of Valladolid.

INVESTIGATOR'S MANUAL.

General protocol:

1. Culture of MSV cells with GMP methodology of the Cell Therapy Unit of the IBGM to manufacture the mesenchymal cell product (for administrative purposes drug) released at 3-4 weeks in a suspension with 25106 ± 5x106 in 2 mL of Ringer-lactate isotonic medium with 0.5% human albumin and glucose 5mM and a cell viability ≥ 80%, loaded into a syringe and labeled, having proven negative microbiological results for microorganisms (Gram-) and mycoplasma and endotoxin level less than 0.5 EU / mL.

Details about the obtaining donor bone marrow by puncture-aspiration and cell processing

They are described in detail in sections 5.3 and 6.1.6 of the protocol.

2. Injection of 2 mL of the MSV suspension into the nucleus pulposus area of the disc degenerated under radiological control to ensure administration in the correct location.

In the control arm of the test, 2 mL of the 1% mepivacaine in saline solution in paravertebral muscles close to the injury.
3. Evaluate the feasibility, safety and evidence of efficacy of the treatment under subjective, objective and imaging clinical criteria that confirm or deny the improvement of symptoms and improvement of dehydration, as well as its maintenance in the time.

Procedure for intradiscal application of MSV:

The collection, preparation and transport of the MSV will take place in the morning and the cells will be applied within 12 hours of packaging. The patient will complete the procedures administrative procedures corresponding to outpatient admission and treatment, following the following procedure: Antibiotic prophylaxis will be performed with 2 gr of IV cefazolin or 1 gr of IV vancomycin in case of allergy. Cell implantation is carried out under mild sedation and radiological control. The cells are injected by puncturing the disc avoiding neurovascular elements (Konings & Veldhuizen, 1988). After double brushing the region with an aqueous solution of povidone-iodine (or chlorhexidine in patients allergic to iodine), the field is delimited with sterile sheets and local anesthesia (Scandicain 1%) to the skin, subcutaneous tissue and muscle near the puncture.
With anteroposterior fluoroscopy, a line is marked on the skin vertical corresponding to the projection of the spinous process with a dermographic pencil sterile. Then a perpendicular line is drawn corresponding to the projected image of a Kirschner wire aligned with the intervertebral space to be treated and a 20G spinal needle tilted 25 to 35 degrees toward the midline at one point located 8 to 9 cm (depending on the patient morphotype) from the midline. In this moment, fluoroscopy is switched to the lateral position to ensure that penetration of the needle follows the correct direction until the nucleus pulposus is reached. After verify the correct position of the needle in the nucleus pulposus both in the fluoroscopic view antero-posterior and lateral, the suspension of cells is injected slowly inject slowly. Using this procedure, we had no incidents in any of the 10 patients from our previous study (Orozco et al., 2011).

Post-treatment guidelines

The patient is discharged after a 2-hour observation period. Not prescribed Lumbostat corset and walking is allowed for moderate periods. The work activity for a week. Exercises are prescribed to tone the paravertebral muscles and abdominals, which begin one month after the intervention. Medication analgesic is adapted to the needs of each patient and use should be avoided as far as possible of anti-inflammatories. Long-term follow-up will consist of clinic visits and radiographic and MR monitoring as specified in section 7.2 of the protocol ("Trial Schedule").

Concomitant treatments

Relevant concomitant medications taken by the patient will be recorded in the CRD regularly due to other parallel treatments allowed during the study. During treatment and subsequent follow-up, corticosteroids should be avoided as much as possibleas well as anti-inflammatory therapy. If it is considered necessary to administer them, you should be noted in the clinical history and CRD.
Drug accounting
The product under study or medicine is produced individually by starting from the bone marrow from each donor. In all cases, the MSV will be registered and identified produced for each patient at the IBGM.

Management of study medication

The study medication, properly packaged MSV in a thermostatic container, was transported immediately after production to the application center. You have 12 hours of stability at room temperature and is applied in the operating room as an injection into the knee joint.

ANNEX II: PATIENT INFORMATION SHEET
Study Title: Treatment of Lumbar Degenerative Disc Disease with Cells allogeneic mesenchymals (MSV *). * "Ex Vivo" expanded bone marrow progenitor cells with GMP procedure of the IBGM of Valladolid.
EudraCT: 2012-004444-30

Dear Patient:
This fact sheet may contain words that you do not understand. In such case, for Please ask the doctor responsible for the study or the personnel participating in the study to Explain any words or information that you do not clearly understand. When there is understood all the information you will be asked to sign a consent form informed to participate in the study.
As a patient diagnosed with Degenerative Disc Disease (DDL), which is a degeneration or "wear" of the spinal disc, suffer from back pain and other discomfort rebellious to the usual treatments and whose evolution is presumably towards a greater deterioration, without meaning that this is necessarily linked to persistence or increased symptoms. In addition to the usual treatment methods, which you may already have received, there is a new treatment option that may perhaps achieve restoration, by less partial, the intervertebral disc to a state close to normal with relief of the symptom. This new treatment consists of the injection into the intervertebral disc of mesenchymal stem cells obtained from a healthy donor (allogeneic MSV) and expanded "Ex vivo" in the laboratory. MSV treatment will be applied to half of the patients participating in this trial. The other half (control group) will receive a similar injection, but without cells. Whether a specific patient is included in one or the other The group will be determined randomly and will not be made public until the end of the clinical trial. One time after the clinical trial, patients in the control group will be offered the possibility of receive mesenchymal cell treatment if it proves to be more effective than control.
The goal of this study, authorized by the Clinical Research Ethics Committee of the Hospital Clínico Universitario de Valladolid and controlled by the Spanish Agency for Medicines and Health Products, is to determine the viability, safety, and indications efficacy of the new therapeutic proposal.
A total of 24 patients will participate in this study. If you decide to participate, it will be required carry out the stipulated tests and procedures that are necessary for the treatment in the assigned group, as well as clinical and radiological follow-up visits that have been scheduled on specific dates and that, in the interests of the reliability of the investigation, must be respected.

Visiting program
The patient visit program will be as follows:
Pre-Inclusion: Patients who come to the consultation and whose diagnostic orientation is based on in the medical history and complementary examinations suggest that it would be beneficial their inclusion in the clinical trial (CT), presenting all the inclusion criteria and none exclusion, they will be prescribed the treatment schedule according to the norm and then They will be informed of the possibility of inclusion in the EC. They will be given the "S*heet of Information to the Patient*" and, if their decision is affirmative, the procedures for inclusion in a next visit.
Inclusion (Visit 0): May require more than one visit. If the patient decides to join the trial you will be asked to sign the "Informed Consent Document". Will be requested then new radiographic and Magnetic Resonance Imaging (MRI) scans with the mandatory characteristics of the study, except if these examinations had already been carried out within a period of no more than 6 months. The analysis and determinations in sera will be carried out regulations that include determination of HIV, Hepatitis and Lúes. In the case of women at childbearing age, a urine sample will also be requested to perform a test of pregnancy. The low back pain evaluation questionnaires (VAS), disability will be passed (Oswestry) and quality of life (SF-12). The MRI will be performed and

scheduled on the day of the intervention (V1). It will be verified that all the inclusion criteria are met for participate in the study, in which case the visit for the intervention will be scheduled. The observer agency (CRO) will determine by a random procedure if you will enter the control or experimental group, and this allocation will remain secret from the patients until the end of the clinical trial.
Visit 1 (day 1): In it, the cultured cells will be implanted (or the hydrogel) in the diseased intervertebral disc, following the procedure similar to an injection.
Follow-up visits (2 to 5): Must be presented for controls at 8 days and at 3, 6 and 12 months after the intervention, in which the clinical, analytical and medical controls will be carried out. image agreed in the protocol. At the end of this period, the analysis of the data and obtaining conclusions from the study, but the medical team that has treated you will remain at your disposal for long-term follow-up and follow-up with other treatments if necessary. The patient is expected to undergo the program of follow-up specified in the preceding paragraphs for a period that will last approximately one year after trial treatment. Subsequently, the patient You must report any incident that could be related to the clinical trial.

Withdrawal from the study

Your participation in this study is completely voluntary, and you may withdraw from it at any time moment, without having to give explanations and without compromising in any way the treatment that you have to receive. The study doctor may also withdraw you from it at any time if you consider it is in your best interest or that it does not meet the necessary requirements to participate because it requires some treatment not allowed in the study, if you do not follow the instructions, if you are a woman and stay pregnant or if the study is canceled. The Essay Promoter may also interrupt it ahead of schedule if they deem it necessary.

Possible risks and drawbacks

As with all effective medications or treatments, some unwanted effect, which we consider to have a very low probability of Present. We consider that the risks of your treatment are no greater than those that involves the usual discography technique, which involves percutaneous puncture of the disc spinal cord under radiographic control and that includes the risk associated with sedation or anesthesia, infections, bleeding, etc.
The proposed treatment is novel, since no treatment has been carried out to date with Allogeneic mesenchymal cells for lumbar degenerative disc disease. As in all research studies, there may be short-term and long-term risks, some known and others that are still unknown, but we do not expect any problem from new health for participating in this study. The main risks that we can imagine are:
At the local level, the disc injection has a small risk of causing bleeding, inflammation or even infection, which should be controllable by procedures usual.
The most serious risks could arise from immune reactions caused by the allogenicity of MSV, causing either rejection of the implanted cells or aggression phenomena against host cells. It is known, however, that mesenchymal cells are highly immune tolerant and unlikely that give rise to these phenomena. On the other hand, mesenchymal cells have been used in several previous clinical trials, usually to treat autoimmune diseases.
In the previous experiences published so far, no Noteworthy Adverse Events Associated with Treatment. I actually know has reported the opposite therapeutic effect, inducing immune tolerance, even in these autoimmune disease patients, in which the balance of the immune system can easily fall apart. For these reasons we think that the problems derived from Immune reactions are very unlikely. It should be emphasized, however, that, as the mesenchymal cells have so far been applied in a limited number of cases, Rare effects may appear that are unknown to us at this time. On In any case, if you experience any problem during the study, you must communicate it immediately to the responsible doctor.

In rare cases when a nurse, doctor, or lab technician is exposed to a patient's blood, tissue, or body fluid due to a needle stick needle, cut, or splash, such blood, tissue, or body fluid may need to be tested to determine the possible existence of certain viral infections, such as hepatitis B and C and HIV This test would allow that person to receive appropriate counseling, and the treatment if necessary. In these cases, the study doctor will ask you to undergo any necessary diagnostic test (usually a blood test), provide information relevant to your health and advise you on next steps. The confidentiality of your data will be respected at all times.
During the development of this clinical trial, medication with corticosteroids and anti-inflammatories, as these treatments can interfere with therapy cellular or complicate the interpretation of your results. If it is necessary to implement these treatments, will be done in the shortest possible way.
It must also be accepted that there may be drawbacks that make it impossible to cell transplantation such as fortuitous failures in the laboratory, growth of pathogenic germs on the cell product, accidents during transportation or situations Similar. If this undesirable and unlikely situation happened, you would be offered to repeat the process.

New information

In the event that important new information appears during the course of the trial about the study treatment you have received, your doctor will inform you adequately about the implications it may have on your participation in the same.

Study review and approval

The performance of this study and all its details has been reviewed and approved by the Ethical Committee for Clinical Research of the Hospital Clínico Universitario de Valladolid and by the pertinent Health Regulatory Authorities.

Confidentiality

Access to your clinical-care data will be done keeping the strictest confidentiality so that personal privacy is not violated. Your data will be treated as so that the information obtained cannot identify you or be associated with you.
In this way you will not be able to be identified during the analysis, in the presentation of the results of the study or in publications related to the study.
If you agree to participate in the study, you authorize that in addition to the doctor and his team, your medical records are reviewed by authorized staff of the Study Sponsor, by the organization designated by the Sponsor to coordinate the study (CRO), by the Regulatory Health Authorities and representatives of the Research Ethics Committee Clinic that has approved the study. During the study, you are guaranteed strict compliance with the Law of protection of personal data (in Spain, Law 15/1999 of December 13, Personal data protection). In any case, you can withdraw consent informed for the processing of your personal data by contacting the researcher.

Gratuitous character

All trial-related procedures, tests, and visits that are carried out will not entail any cost since it is covered by funds for aid to the investigation.

Compensation

If, as a result of your participation in the trial, you present some type of adverse reaction to the medication or if the study has effects on your health, the research team study doctors will provide the necessary medical care and notify the Promoter. All patients participating in the study will be insured according to the applicable and current national and international legal requirements. The Promoter of the study, claims to have subscribed an insurance policy with the company HDI HANNOVER INTERNACIONAL with the number 1301001/008413, for civil liability, which covers the damages that could result as a consequence of the clinical trial in the person or persons to be carried out, in accordance with the provisions of the current legislation.
In no way, the fact of signing the informed consent means that you lose or waives the legal rights that may correspond or exempts the researchers, the promoter of the study

and the institutions involved in it of the obligations and corresponding professional responsibilities.

Acknowledgwments

Thank you very much for reading this clinical trial information document. Please take enough time before deciding if you want to participate. If you decide to take part in the study, please sign the informed consent form. You will be given a copy of this informative document and the consent document for you to save.

If at any time during the study you would like to ask a question regarding the treatment or your condition, or in case of any emergency, do not hesitate to contact contact with:

Dr …….. ……. Telephone ……

Dr …….. ……. Telephone …….

INFORMED CONSENT DOCUMENT

Study Title: Treatment of lumbar degenerative disc disease with mesenchymal cells allogeneic (MSV *).* "Ex Vivo" expanded bone marrow progenitor cells with the GMP procedure of the IBGM of Valladolid

EudraCT: 2012-004444-30. Code: Disc_allo_MSV

I ……. (Name and surname).

I declare that:

I have read the information sheet that has been given to me.

I have been able to ask questions about the study.

I have received enough information about the study.

I have spoken to..... ..... (Investigator's name)

I understand that my participation is voluntary and that I can withdraw from the study when want, without having to give explanations and without this having an impact on my medical care.

Having carefully examined all the circumstances, I freely lend my compliance to participate in the study.

....

(date) (participant's signature)

....

(date) (researcher's signature)

ALTERNATIVE CONSENTS

ORAL CONSENT DOCUMENT BEFORE WITNESSES

Study Title: Treatment of lumbar degenerative disc disease with mesenchymal cells allogeneic (MSV *). * "Ex Vivo" expanded bone marrow progenitor cells with the GMP procedure of the IBGM of Valladolid EudraCT: 2012-004444-30. Code: Disc_allo_MSV

Me,..... ....., (name and surname) I declare under my responsibility that .... (Name of trial participant):

You have received the information sheet about the study.

You have been able to ask questions about the study.

You have received enough information about the study.

Has been informed by .... (name of the researcher).

You understand that your participation is voluntary and that I may withdraw from the study when you want, without having to explain and without having an impact on your medical care.

Once all the circumstances have been carefully examined, he freely lends his compliance to participate in the study.

....

(date) (witness signature)
....
(date) (researcher's signature)

REPRESENTATIVE's CONSENT DOCUMENT
Study Title: Treatment of lumbar degenerative disc disease with mesenchymal cells allogeneic (MSV *).
*** "Ex Vivo" expanded bone marrow progenitor cells with the GMP procedure of the IBGM of Valladolid**
EudraCT: 2012-004444-30. Code: Disc_allo_MSV
Me,..... .... (Name and surname)
acting as .... (relationship with the participant)
from .... (name of trial participant)
I declare that:
I have received the information sheet on the study.
I have been able to ask questions about the study.
I have received enough information about the study.
I have been informed by .... (name of the researcher).
In my presence it has been given to .... (name of participant) all the information pertinently adapted to your level of understanding and he agrees to participate.
And I agree that .... (participant's name)
Will participate in this study.
....
(date) (representative's signature)
....
(date) (researcher's signature)

DOCUMENT OF CONSENT OF DONATION OF CELLS FOR REEXPANSION, REIMPLANTATION OR INVESTIGATION
PLEASE NOTE THAT THIS IS THE INFORMED CONSENT FOR THE DONOR of the Disc_allo_MSV trial
(Checkmark as appropriate)
-As a patient included in the clinical trial: "…… .. EudraCT …… ..., Code….
-As a patient included in the Study "…… ..... EudraCT: …… Code….
In the event that after the cultivation process a greater number of cells is obtained than the established to be implanted, I accept that:
Leftover cells can be preserved and manipulated as convenient to carry out, indistinctly and in this order, 1) reapplication of cells for myself if necessary and convenient, 2) allogeneic application in another patient who could benefit from them or 3) use in the projects of research carried out by the Cell Production Unit at the facilities of the Institute of Molecular Biology and Genetics or the Science Park of the University of Valladolid. Once these uses have been carried out or when the cells are not useful will be destroyed following the standard procedures in this Unit of Production.
I declare that I have understood the information given to me in this regard and that I have been had clarified all the questions or doubts that I have wanted to raise, so I freely take the decision to authorize the use of surplus cells, understanding that:

 All personal information will be treated confidentially and encrypted for the protection of my identity
 This donation is altruistic and I will not receive any compensation for it
 I am exempt from any liability that may arise from the use of these cells

Place and Date: …… ..to …… ..of… ..… ..… ..… ..… ..of 201…

NAME AND SURNAME OF PATIENT: …….
……
ID Number (Passport): …….. SIGNATURE:

RESPONSIBLE PERSON THAT PROVIDES THE INFORMATION:
NAME AND SURNAME:
DNI: SIGNATURE

CELL DONATION CONSENT DOCUMENT FOR ALLOGENIC IMPLANTATION.
PLEASE NOTE THAT THIS IS INFORMED CONSENT FOR DONORS
ALTERNATIVES, NOT Included in the MSV_artro trial
Me,..... .... (Name and surname), with DNI Num …… ..that I will be subjected to an intervention of ……. at the Hospital Cínico Universitario de Valladolid scheduled for the day …… I have been informed by .... …… .. (Name of researcher) the possibility of donating a bone marrow sample, which would be obtained during the surgical intervention to which I will be subjected. This sample will be used to obtain Mesenchymal stem cells for allogeneic transplantation in the clinical trial entitled "Treatment of lumbar degenerative disc disease with allogeneic mesenchymal cells (MSV) ". The cells will be handled in the existing White Room at the facilities of the Institute of Molecular Biology and Genetics and the Science Park of the University of Valladolid. If there is a surplus, I authorize its use for the projects of research carried out at these facilities. Once these uses or when the cells are not useful they will be destroyed following the procedures standardized in this White Room.
I declare that I have understood the information that has been provided to me in this regard and that it have clarified all the questions or doubts that I wanted to raise, so I take freely decide to authorize the use of my bone marrow cells for purposes mentioned above, understanding that:
 All personal information will be treated confidentially and encrypted for the protection of my identity
 This donation is altruistic and I will not receive any compensation for it
 I am exempt from any liability that may arise from the use of these cells
Place and Date: …… ..to …… ..of… ..… ..… ..… ..… ..of 201…
Patient Signature: Investigator Signature
DNI: …… .. DNI: …….

ANNEX III: HELSINKI STATEMENT

HELSINKI STATEMENT OF THE WORLD MEDICAL ASSOCIATION
Ethical Principles for Human Medical Research
Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964 and
amended by 29th World Medical Assembly, Tokyo, Japan, October 1975
35th World Medical Assembly, Venice, Italy, October 1983
41st World Medical Assembly, Hong Kong, September 1989
48th General Assembly Somerset West, South Africa, October 1996
52nd General Assembly, Edinburgh, Scotland, October 2000
Clarification Note on Paragraph 29, WMA General Assembly, Washington 2002
Clarification Note on Paragraph 30, WMA General Assembly, Tokyo 2004
59th General Assembly, Seoul, Korea, October 2008

ANNEX IV. NOTIFICATION OF SEVERE ADVERSE EFFECTS

NOTIFICACION DEOF SUSPICION OF ADVERSE REACTION FOR IMP

	2. FECHA DE NACIMIENTO			2a. EDAD	3. SEXO		3a.	3b.	4-6. FECHA
	DIA	MES	AÑO		< HOMBR			DÍA	MES AÑO
7. DESCRIPCIÓN DE LA REACCIÓN ADVERSA (Incluvendo resultados relevantes							8-13b. CRITERIOS DE GRAVEDAD/ DESENLACE		

I. INFORMACION SOBRE LA REACCIÓN ADVERSA

II.

< LA VIDA DEL PACIENTE HA ESTADO EN PELIGRO

< HOSPITALIZACIÓN

< PROLONGACIÓN HOSPITALIZACIÓN

< INCAPACIDAD PERMANENTE O SIGNIFICATIVA

< RA CLINICAMENTE RELEVANTE

< PERSISTENCIA DE LA REACCIÓN ADVERSA

< RECUPERACIÓN

O	15. SIS		DIO	18. FECHAS DE INICIO	19. DURACI

20. ¿REMITIÓ LA REACCIÓN AL SUSPENDER LA MEDICACIÓN?	20a. ¿REMITIÓ LA REACCIÓN AL REDUCIR LA DOSIS?	21. ¿REAPARECIÓ LA REACCIÓN AL ADMINISTRAR DE NUEVO LA MEDICACIÓN?

II. INFORMACION DEL MEDICAMENTO EN INVESTIGACIÓN

III. MEDICAMENTOS CONCOMITANTES E HISTORIA CLÍNICA

23. DATOS IMPORTANTES DE LA HISTORIA CLÍNICA (ej. diagnósticos, alergias, embarazos, etc.)

IV. INFORMATION ON THE SPONSOR

24a. NOMBRE Y DIRECCION DEL PROMOTOR		24b. NOMBRE Y DIRECCION DEL INVESTIGADOR
24c. CODIGO DE LABORATORIO (Nº AEM)	25a. TIPO DE INFORME <INICIAL <SEGUIMIENTO	24c. TECNICO DEL PROMOTOR QUE INFORMA NOMBRE: TELEFONO: FIRMA:
24e. FECHA DEL INFORME	24f. FECHA DE ENTRADA AEM	25b. SE ADJUNTA INFORME COMPLEMENTARIO

GENERAL INSTRUCTIONS

1. This form will only be used to report suspected adverse reactions (AR) serious and unexpected occurrences with investigational drugs. It is considered medicine under investigation, both the specifically investigated product and the control.
2. Suspected fatal or life-threatening adverse reactions (those that are not having mediated an immediate therapeutic intervention would have meant the death of the patient)

They will communicate within a maximum period of 7 calendar days; if not all the information is available, this it can be completed within an additional 8 days. Other suspected adverse reactions serious and unexpected will be communicated within a maximum period of 15 days.
3. When the available space is insufficient, an additional information sheet will be added, correctly identified with the name of the promoter and the number assigned to the notification. In this Additional information may include the evaluation of causality carried out by the technician that informs.

SPECIFIC INSTRUCTIONS
1. The protocol code is the one assigned by the promoter to identify the assay. The number of the promoter notification is the one that the promoter uses for its file. When it comes to information from the tracking number will be used or, if it is modified, the number of the initial notification. The "Notification No." space that appears shaded will be left unfilled.
2. The age will be set in years, months, weeks or days as appropriate, but always indicating it. the age is not precisely known, it must refer, at least, to the age group to which it belongs (p. eg: infant, child, adolescent, adult, elderly).
3. The adverse reaction will be described in full, indicating the end date of the itself and including the results of complementary examinations or laboratory tests that are considered of interest. This notification may be accompanied by as many reports as deemed convenient for the proper interpretation of the clinical picture suspected of being a reaction adverse.
4. The categories are not mutually exclusive. Assistance in an Emergency Service of a Hospital less than 24 hours, hospitalization will not be considered.
5. Investigational drugs will be identified if possible by their generic name (DOE or DCI), indicating when the commercial name is available, or failing that, by the name proposed or laboratory code for the product.
6. In the event that the administration is not daily, an attempt will be made to describe it with one of the following possibilities: cyclical, weekly, monthly, yearly or number of times it has been used (putting in in this case the dose of each dose, not the total).
7. The pathological process of the patient to whom the product is intended shall be recorded in research, or "healthy volunteer" in case of such.
8. The duration of treatment until the beginning of the adverse reaction will be stated.
9. It will be explicitly stated if concomitant drugs have not been taken. In the case of considering suspicious one or some of the concomitant drugs will be marked with an asterisk (e.g .: * AMOXICILLIN). Medications used to treat the adverse reaction will be excluded.

ANNEX V. Visual analog scale (lumbar and sciatic pain)
Visual Analog Scale (VAS)
NAME AND SURNAME:
Date
Measurement of back pain (low back pain) in your daily life:
Assess your current pain while doing activities of your daily life in the last 4 weeks, putting a vertical mark on the horizontal line that we present to you.
The left side represents the absence of pain and the right, excruciating pain.

(No pain) Maximum pain

Measurement of leg pain (sciatic pain) in daily activities.
Assess the sciatic pain that you present today when carrying out activities of your daily life in the last 4 weeks, putting a vertical mark on the horizontal line that we present.
The left side represents the absence of pain and the right, a pain unbearable.

(No pain) Maximum pain
Thank you very much for your help.

ANNEX VI: OSWESTRY DISABILITY INDEX

Name and surname:…….
Date:……..
In the following activities, mark with a cross the phrase that in each question is more closely match your situation:

1.Intensity of pain
or (0) I can bear the pain without needing to take painkillers
or (1) The pain is strong but I manage without taking painkillers
or (2) Pain relievers completely relieve my pain
or (3) Pain relievers give me some pain relief
or (4) Pain relievers hardly relieve my pain
or (5) Pain relievers do not relieve my pain and I do not take them

2 stand up
or (0) I can stand as long as I want without increasing pain
or (1) I can stand as long as I want but it increases the pain
or (2) Pain prevents me from standing for more than an hour
or (3) Pain prevents me from standing for more than half an hour
or (4) Pain prevents me from standing for more than 10 minutes
or (5) Pain prevents me from standing

3.Personal care
or (0) I can manage on my own without increasing pain
or (1) I can manage on my own but this increases the pain
or (2) Washing, dressing, etc., causes me pain and I have to do it slowly and with watch out
or (3) I need some help but I manage to do most of the things myself
or (4) I need help to do most of the things
or (5) I can't get dressed, I find it hard to wash and I usually stay in bed

4.Sleep
or (0) Pain does not prevent me from sleeping well
or (1) I can only sleep if I take pills
or (2) Even taking pills I sleep less than 6 hours
or (3) Even taking pills I sleep less than 4 hours
or (4) Even taking pills I sleep less than 2 hours
or (5) Pain totally prevents me from sleeping

5.Lift weight
or (0) I can lift heavy objects without increasing pain
or (1) I can lift heavy objects but it increases the pain
or (2) Pain prevents me from lifting heavy objects off the floor, but I can do it if they are in a comfortable place (e.g. on a table)
or (3) Pain prevents me from lifting heavy objects, but I can lift objects light or medium if they are in a comfortable place
or (4) I can only lift very light objects
or (5) I cannot lift or lift any object

6.Sexual activity
or (0) My sexual activity is normal and pain is not increasing
or (1) My sexual activity is normal but the pain increases
or (2) My sexual activity is almost normal but the pain increases a lot
or (3) My sexual activity has been severely limited due to pain
or (4) My sexual activity is almost nil because of the pain
or (5) Pain prevents me from all kinds of sexual activity

7.Walk
or (0) Pain does not prevent me from walking
or (1) Pain prevents me from walking more than one kilometer

or (2) Pain prevents me from walking more than 500 meters
or (3) Pain prevents me from walking more than 250 meters
or (4) I can only walk with a cane or crutches
or (5) I stay in bed most of the time and have to crawl to the bathroom

8.Social life

or (0) My social life is normal and the pain does not increase
or (1) My social life is normal but it increases the pain
or (2) The pain does not have a significant effect on my social life, but it does prevent my more energetic activities like dancing, etc.
or (3) Pain has limited my social life and I don't go out as often
or (4) Pain has limited my social life to home
or (5) I have no social life because of the pain

9. Sitting

or (0) I can sit in any type of chair for as long as I want
or (1) I can sit in my favorite chair for as long as I want
or (2) Pain prevents me from sitting for more than an hour
or (3) Pain prevents me from sitting for more than half an hour
or (4) Pain prevents me from sitting for more than 10 minutes
or (5) Pain prevents me from sitting

10.Travel

or (0) I can travel anywhere without increasing pain
or (1) I can travel anywhere, but it increases the pain
or (2) The pain is strong but I can endure trips of more than 2 hours
or (3) Pain limits me to trips of less than an hour
or (4) The pain limits me to short and necessary trips of less than half an hour
or (5) Pain prevents me from traveling except to go to the doctor or hospital

Thank you very much for answering the questionnaire

ANNEX VII: SF-12 HEALTH QUESTIONNAIRE
SF-12v.2 HEALTH QUESTIONNAIRE (Short version of SF-36)
(Gandek B, Ware JE, Aaronson NK, Apolone G, Bjorner JB, Brazier JE, Bullinger M, Kaasa S, Leplege A, Prieto L & Sullivan M. (1998). Cross-validation of item selection and scoring for the SF-12 Health Survey in nine countries: results from the IQOLA Project. International Quality of Life Assessment. J Clin Epidemiol 51, 1171-1178.)

ANNEX VIII. MODIC CHANGES
MODIC CHANGES.
Described by Modic in 1988 is a common and usual observation in a projection of magnetic resonance imaging, are changes in signal intensity in the spinal cord bone of the vertebra in the area adjacent to the ends of the vertebral discs degenerative.
They represent different histological states of the subchondral bone marrow.
It is common to find these changes in patients with nonspecific low back pain and is associated with pain. However, it should be noted that these changes may be present in the individuals without low back pain.
Modic I:
Corresponds to inflammatory changes with neovascularization, which determine prolongation of T1 and T2 with decreased signal in T1 and hypersignal in T2. Histologically it is correlates with microfractures and edema in the vertebral platforms, in addition to tissue vascularized granulatory fibrous, such that changes can be demonstrated with use gadolinium. These alterations are reversible or can progress to stages II and III.
Modic II:
Corresponds to degenerative fat infiltration and bone demineralization of the bone spongy subchondral. Consequently, there is shortening of T1 with increased signal in T1-weighted sequence and T2 prolongation with increased signal in sequences boosted in T2.
Modic III:
It corresponds to the so-called "discogenic sclerosis" that is observed in plain radiography in vertebral platforms and is due to regeneration with bone remodeling subchondral, which determine T1 prolongation and T2 shortening with hyposignal in T1 and T2 weighted sequences
In summary, the signs of MRI are:
 TYPE I T1 low T2 rises = edema
 TIP0 II T1 rises T2 equal or rises = fat degeneration
 TYPE III T1 low T2 low = bone sclerosis