ADIPOA Report Summary

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Final Report Summary - ADIPOA (ADIPOSE DERIVED STROMAL CELLS FOR OSTEOARTHRITIS)

Executive Summary:

Among the degenerative diseases associated with aging, osteoarthritis is the most common pathology and affects 16% of the female population over 65 years. The ADIPOA project started in January 2010 with the goal to develop a new cell based strategy for patients suffering from knee osteoarthritis (OA). Up to now, no therapeutic option exists to obtain a sustainable improvement of joint function beside knee arthroplasty. This prompted us to propose adipose derived stem cells as a possible cell therapy.

Mesenchymal stem cells are known to have a potential for articular cartilage regeneration and anti-inflammatory properties. However, most studies focused on focal cartilage defect through surgical implantation. For the treatment of generalized cartilage loss in osteoarthritis, an alternative delivery strategy would be more appropriate. The purpose of this study was to assess the safety and efficacy of intra-articular injection of autologous adipose tissue derived MSCs (ADSCs) for knee osteoarthritis. In this pilot open study, the clinical outcomes and MRI findings of intra-articular injection of autologous adipose derived stem cells for treatment of patients with severe knee osteoarthritis (OA) were evaluated.

Methods We enrolled 18 patients with osteoarthritis of the knee and injected AD MSCs into the knee. The phase I study consists of 3 dose-escalation cohorts; the low-dose (1.0x10^7 cells), mid-dose (5.0x10^7) and high-dose (1.0x10^8) group with 6 patients each. The primary outcomes were the safety and the Western Ontario and McMaster Universities Osteoarthritis index (WOMAC) at 3 months. Secondary outcomes included clinical, radiological, and histological evaluations. Stem cell injections were administered to 18 patients (mean 65 years) with severe knee OA (KL 3 60%, KL 4 40%) with long disease duration (mean 10 years) after informed consent. All patients were symptomatic despite analgesics treatment and required arthroplasty. Subcutaneous adipose tissue was harvested from abdominal fat by liposuction. After stromal vascular fractions were isolated, ADSC were expanded for 2 weeks in GMP condition in the presence of platelet extract. ADSC were characterized by the presence of CD73, CD105, CD90 markers, and viability above 85%. For the first group (low dose) 6 patients, 2 10^6 cells were injected in the symptomatic knee joint under control of echography. The second group (medium dose) of patient received 10 10^6, and the last group (high dose) of 6 patients 50 10^6 ADSC. Outcome measures included the Knee Injury and Osteoarthritis Outcome Scores, visual analog scale (VAS), and WOMAC score at preoperative and 1-, 3- and 6-month follow-up visits.

Results: Almost all patients showed significant improvement in all clinical outcomes at the final follow-up examination in the first group of patients. We observed a significant % improvement (WOMAC decrease of 40%, KOOS increased of 30%, and VAS improved of 50%) in 80 patients at 3 month follow-up compared to baseline (P<0.05). In the medium group of patients, improvement was less significant. At last, in the high dose group, 2 patients experienced transient knee joint pain and swelling after local injection. The procedure was safe, and no SAE was reported. Moreover, only 2 of the patients underwent total knee arthroplasty during the 1-year follow up period.

Conclusion Adipose-derived stem cell therapy for patients with severe knee OA was safe and feasible, reducing pain, and improving function in the low dose group. However, randomized controlled trial is warranted before conclusion on efficacy can be taken. The ADIPOA research teams performed successfully the phase 1 clinical trial is in France and Germany. A phase 2B
controlled trial is scheduled to confirm the clinical benefit of this strategy.

Project Context and Objectives:

Osteoarthritis (OA) is the most common form of human arthritis. The disease is characterized by degeneration of the articular cartilage, with loss of matrix, fibrillation, formation of fissures, and ultimately complete loss of the cartilage surface. OA is a clinical syndrome of joint pain accompanied by varying degrees of functional limitation and reduced quality of life (Woolf AD et al). Knees, hips and small hand joints are the joints most commonly affected. Key pathological changes in OA include localized loss of articular cartilage and remodelling of adjacent bone with new bone formation at the joint margins. This combination of tissue loss and new tissue synthesis supports the view of OA as a joint repair process; the process is slow but efficient, and often compensates for the initial trauma, resulting in a structurally altered but symptom-free joint. OA usually has a slow and insidious onset and affects only one or a few joints. The most important symptoms are pain and limitation of motion. Although the pain is an annoyance that can curtail daily activities, the limitation of motion can translate into loss of function. Signs of OA include tenderness and the development of firm swellings around the joint margins. Bony enlargement of the affected joint(s) and crepitus are signs that help physicians differentiate OA from other rheumatic disorders. In clinical practice, the diagnosis of OA is primarily based on history and physical examination supplemented by plain X-rays of the affected joint. Where standardized diagnostic criteria are required, the mostly widely used are those developed by the American College of Rheumatology (ACR) (Altman R et al). Key radiologic features of OA include joint space narrowing and bony proliferation with the presence of osteophytes/spurs. In routine clinical practice, radiological severity of OA is largely assessed based on radiologists’ judgement; in clinical trials and other research, use is made of the Kellgren-Lawrence (0-4) grading scale. The relationship between radiographic structural change and pain and function in OA is not strong.

MSCs have been isolated from the surface zone of articular cartilage and synovial tissue and MSCs have the capacity to repair fibrillated cartilage in the goat OA model. It is attractive to hypothesize that ASC could have a role in cartilage protection by secreting growth factors, by a direct resurfacing of the articular cartilage or act to preserve subchondral or trabecular bone structure-associated mechanical integrity of the joint. MSCs injected into the knee joint did not bind to normal or fibrillated articular cartilage in vivo, but prevented dramatically OA progression in the goat model by an effect other than that seen on meniscal regeneration. We showed that MSC-based repair in the presence of hyaluronan may therefore accelerate and amplify the natural repair process of chondrocytes and may contribute to the formation of new cartilage and meniscus after meniscectomy.

In the rabbit (ACLT) model of OA we have also obtained a significant reduction in OA progression, by the delivery of MSCs loaded on a degradable hyaluronan scaffold (figure 2). Furthermore there are several examples of the use of stem cells for articular cartilage repair were the cells have been delivered to either cartilage or bone using a 3-dimensional scaffold that is fixed to the defect site, usually by means of an open surgical procedure. There are many issues associated with the selection of the scaffold material, including its ability to support cell viability and differentiation and its retention and degradation in situ. However, with the aim to limit the surgical intervention and to decrease the cost of this treatment, we will use a simple, scaffold-free approach in which the cells are delivered as a suspension by direct intraarticular injection. The suspension will be prepared in a dilute solution of sodium hyaluronan, which is commonly used for the treatment of OA and also has the effect of increasing the chondrogenic activity of MSCs. We have shown in this goat model of OA, that mesenchymal stromal cells induced a marked regeneration of the joint tissue and a clear reduction in OA progression in the cell-treated joints. We will use both OA models (goat and rabbit) to assess safety, dose response and efficiency of ASC injected in the knee joint after meniscectomy. The use of mouse model of OA presents the advantages of a large panel of reagents and techniques available to look insight the mechanism of the improvement in OA progression by the delivery of ASCs.

Different murine models are described, that allows studying early events occurring after ligaments resection or cartilage injury leading to OA. As an example, the collagenase injection in the knee joint of mice results in proteoglycan loss, expression of epitopes reflecting release of MMPs and osteophyte formation and synovial thickening (Scharstuhl A et al). Crucial ligament section in the DMM model results in OA like lesions after 6 weeks by histological evaluation and both the cartilage and bone can be morphometrically characterized using confocal laser scanning microscopy (CLSM) and micro-computed tomography (µCT). These imaging approaches allow the quantitative analysis of morphometric changes by the measures of cartilage thickness and volume as well as trabecular and cortical bone parameters (Blaney Davidson EN et al). These murine models are appropriate models to assess biological effect of ASC and the role of secreted growth factors.
Differentiation of stromal progenitor cells to osteoblast, chondroblast or adipocyte are tightly regulated by transcription factors including AP1, runx 2, sox family members. AP-1 is an heterodimeric transcription factor formed by the association of one of the four members of the Fos proteins (c-Fos, FosB and its isoform DFosB, Fra-1 or Fra-2) with one of the members of the Jun family (c-Jun, JunB or JunD). AP-1 activity is regulated by most changes affecting the cellular environment thereby regulating cell differentiation, cell death and differentiation. Of interest are the key roles of AP-1 in controlling mesenchymal cell fate decision, particularly in the development of bone, cartilage and adipose tissues. Indeed, genetically modified mice have demonstrated that over-expression of c-Fos was leading to the development of osteochondrosarcomas caused by the transformation of the osteochondroprogenitor cells. The transgenic mice over-expressing DFosB, Fra-1 or Fra-2 are all developing osteosclerosis due to cell autonomous increased differentiation of the bone forming cells or osteoblasts. In addition, Fra-2 was shown to be essential for chondrocyte differentiation and mGlap, a matrix protein secreted by chondrocytes was identified as Fra-1 target gene. Interestingly, in both DFosB and Fra-1 transgenic mice the increased bone formation was paralleled by a progressive lipodystrophy due to a cell autonomous defect in adipocyte differentiation. Interestingly, most of the so far identified AP-1 target genes are encoding for proteins involved in extracellular matrix remodelling (component of the matrix and enzymes that digest it). Fra1 Transgenic mice were used to induce cartilage lesion mimicking OA through collagenase intra-articular injection. In these animals, the cartilage thickness and volume were significantly reduced and increased, respectively, in the joints receiving collagenase treatment whereas no significant changes could be measured when MSC expressing AP1 were implanted, suggesting a strong protective effect of mesenchymal stromal cells (unpublished results). These models are available by one Participant and will offer an insight in the biology and improve our knowledge on how ASC prevents OA and intrinsic difference between ASC wt and Fra1 Transgenic mice.

The ADIPOA Consortium comprises 13 Participants involved in 4 activities (work packages 1-4) with 2 additional WP (WP5 & WP6) for coordination and training. Its final aim is to provide Europe with a solid scientific basis on the use of ASC in order to prevent and treat osteoarthritis. The WPs were the following:

- WP 1 ASC biology, cell processing & optimization for chondral protection
- WP 2 In vivo validation of chondroprotective effect of ASC
- WP 3 Safety, Security & regulatory issues
- WP 4 Clinical trial endogenous ASC injected intraarticular in OA
- WP 5 Management and Coordination
- WP 6 Training and Education

The first 2 WPs deal with the analysis of the impact on intrinsic cartilage stem cells that may explain chondroprotective effect of ASC. WP1 envision the interactions between transplanted stem cells and host cartilage cells, impact of inflammation and potential ways to circumvent this and. WP 2 focus on in vivo models for preclinical issues, murine OA models to assess mechanism of chondroprotection, including Fra and Ob/Ob transgenic models available, and large animal models to assess dose and safety issues (goat and rabbit OA models) and set up of MRI assessment of GAG content in the cartilage. WP 3 describes procedure to isolate and expand ASC for clinical use, set up original procedure and devices to prepare clinical studies in relation with data from WP1 and WP2 and manage regulatory issues and dossier to obtain approval by EMEA and national agencies and logistics for the clinical applications. WP4 translated these innovations into human medicine through the design and conduction of phase 1 clinical trials and included 18 patients with primary endpoints to assess safety and dose response. Finally, WP5 and 6 were dedicated to the project management and training.

In a first step, we addressed ASCs isolation procedures, the importance of the adequate cell culture conditions (e.g importance of the culture medium used, hyaluronan support). The procedure includes obtaining human samples, dissection and incubation with collagenase, washing, centrifugation and expansion of ASC, potentially using Procore biomed’s GMP-grade FGF2 variant with enhanced activity. We will establish chondrocyte activation and extracellular matrix synthesis and cells proliferation through co culture system with ASC with an optimized ratio. The coculture was performed in transwell, in the presence or not of MatrixCoat as a support. Effect due to direct cell-cell contacts was determined by mixed cultures experiments. For this, we assessed the interactions between ASC and chondrocytes in order to promote the differentiation of later cells and protect
them. Chondrogenic differentiation process is tightly regulated by factors as CTGF BMP2, GDF5, bFGF, Sonic hedgehog, PThr and TGFb1/3, most of them secreted by ASC. We also determined possible interactions between ASC and endogenous cartilage stem cells through HGF secretion by ASC. Cartilage has been shown to include 7% of undifferentiated stem cells, with a pluripotent differentiation potential. Inhibition of hypertrophy and/or apoptosis of OA chondrocytes in coculture will be performed using QPCR and Tunnel assay. For this task, the coculture used primary chondrocytes expanded from OA patients. We assessed chondrocyte induced changes induced by ASC environment by large scale genomic/proteomic analysis. Particularly, chondrocyte phenotype was assessed through QPCR expression of collagen II, aggrecan, Sox-9, COMP, collagen X and FGFR3 expression and extracellular matrix content. We identified HGF as a major component of chondrocyte apoptosis and hypertrophy prevention. In parallel, Analysis of the proteomic expression in ASC indicated PTX and secretin, DKK3, IGF1, CCN3, WISP2 as the putative molecular mechanisms accounting for the chondroprotective potential of the cells.

In a second step, the cellular and biochemical processes identified in the former case were applied in therapeutic models of in vivo repair in OA using collagenase model. One of the major scientific goals of ADIPOA was the understanding of the chondrocyte response to ASC therapy in case of injury and compare with controlled joint with poorly organized, non-functional or scarred cartilage tissue. For this aim we will use both murine and rabbit and caprine mechanical OA models available by our partners that allow the in vivo functionality of ASC therapy. We were able to validate the benefit of ASC therapy and demonstrate safety, dose response. This part focused on the translation of the technology for the development of new therapeutic strategies for ASC and we defined a number of key questions: (1) the levels of engraftment of delivered cells in joint, (2) the trophic effects exerted by the transplanted cells, ie. their ability to contribute repair factors to which the host responds, and (3) the manner in which host cells are mobilized and respond to the presence of transplanted cells.

Third and most important step is the clinical applications, including pilot trial. This clinical open phase 1 trial using clinical grade ASC in symptomatic knee OA was initiated in December 2012. Clinical grade ASC isolation and expansion procedure have been approved by one of the Participant. The clinical study, with a primary end point on feasibility and safety will be initiated after obtaining regulatory approval and ethical committee authorization (November 2011). The study was conducted between months 18 and 36 using autologous ASC focusing on symptomatic OA according to EMEA guidelines. This translation to the clinic was possible through a strong partnership with SMEs and regulatory bodies for the scale-up procedures. Almost all patients showed significant improvement in all clinical outcomes at the final follow-up examination in the first group of patients. We observed a significant % improvement (WOMAC decrease of 40%, KOOS increased of 30%, and VAS improved of 50%) in 80 patients at 3 month follow-up compared to baseline (P<0.05). In the medium group of patients, improvement was less significant. At last, in the high dose group, 2 patients experienced transient knee joint pain and swelling after local injection. The procedure was safe, and no SAE was reported. Moreover, only 2 of the patients underwent total knee arthroplasty during the 1-year follow up period.

Adipose-derived stem cell therapy for patients with severe knee OA was safe and feasible, reducing pain, and improving function in the low dose group. However, randomized controlled trial is warranted before conclusion on efficacy can be taken. The ADIPOA research teams performed successfully the phase 1 clinical trial is in France and Germany. A phase 2B controlled trial is scheduled to confirm the clinical benefit of this strategy.

The strong interactions between teams with complementary expertise in the clinics, MRI and cell therapy will open new perspectives in the field. The final goal of ADIPOA is to develop clinical procedure and scale up facilities using ASC in OA unresponsive to previous medical treatments in partnership with 4 SMEs and regulatory bodies, taking advantage of the experience already acquired by the partners in clinical trials using autologous ASC and chondrocyte. Participant 5 (MABIO) and 12 (PROCORE BIOMED) are already working together to set up device and standardized approved procedure for chondrocyte cell therapy. This phase 2 large multicentre trial will be designed within the 4 year ADIPOA program, but initiated with the help of our partners until the last recruited patients have completed the study. This consortium envision future clinical applications taking advantage of the knowledge gathered on the factors intrinsic to stem cells and on extrinsic factors that are essential for cartilage regeneration.
Project Results:

WP1: Biology, cell processing and optimization for chondroprotection:

Mesenchymal stem cells isolated from bone marrow (MSC) or adipose (ASC) tissues secrete a large amount of trophic factors. The possibility that these cells, through their paracrine potential and their multiple interactions with neighbouring cells, may influence the course of chronic degenerative disorders and prevent cartilage degradation is promising for the treatment of osteoarthritis (OA). This work-package was in charge of investigations on ASC biology, cell processing & optimization for chondral protection.

The first and last tasks (1 and 8) were technological ones to refine GMP cell culture conditions and to set-up ASC purification and expansion in different species in order to gather data on pre-clinical models (murine, rabbit and goat) to ensure the best safety and efficacy of the cell product. The cell culture processes were adapted and permitted investigations on all these models (cf following work-packages particularly WP3).

The second, third and fifth tasks were crucial because their aims were to establish the effects of ASC on chondrocytes in co-culture experiments (partners 1, 3, 4, 11). OA ASCs were isolated from intra-articular (Hoffa-ASC) or hip (hip ASC) subcutaneous adipose tissue and healthy ASCs from subcutaneous abdominal depot (abdo-ASC). The chondrocytes were purified either from normal or OA donors. In parallel, co-culture experiments with synoviocytes were conducted. Different culture models were used including cell culture inserts to avoid cell-cell contact where clinical grade ASCs were co-incubated with other cells in separated compartment.

The effect of ASCs from normal donors was tested on chondrocyte proliferation and apoptosis as well as on the expression of markers specific for fibroblasts or mature and hypertrophic chondrocytes. We demonstrated that ASC-derived factors did not affect chondrocyte proliferation. Nevertheless, when apoptosis was pharmacologically induced in chondrocytes using camptothecin, the number of apoptotic chondrocytes was lower when chondrocytes were cocultured with ASCs compared to chondrocytes cultured alone. After 7 days of ASC or MSC co-cultures with chondrocytes, a stable expression of the markers specific for mature chondrocytes (Collagen IIB, Aggrecan, link, Sox9) was observed, while expression of hypertrophic (MMP13, Alkaline phosphatase, collagen X, RUNX2) and fibrotic (Collagen I, III, VI and vimentin) markers was significantly decreased.

These effects seem different according to the locations of fat sampling. Indeed, compared to abdo-ASCs, Hoffa- and Hip-ASCs reduced less efficiently the expression of hypertrophic and fibrotic markers and some markers of mature chondrocytes (Agg, Col IIB, Sox9) were decreased with Hip-ASCs. In order to identify factors responsible of ASC effects, we quantified by ELISA factors known to be involved in fibrosis and matrix remodelling. The secretions of TIMP-1 and -2, MMP-1 and -9 and IL1-RA were not changed in co-culture. In contrast, secretion of the pro-fibrotic factor TGFβ1 and the anti-fibrotic factor HGF were significantly decreased or induced in co-culture, respectively. Moreover the addition of neutralizing HGF antibody reversed the anti-fibrotic effect of ASCs whereas the hypertrophic markers were not modulated (Maumus et al, 2013). Altogether these results demonstrate that ASCs from abdominal subcutaneous fat were shown to have a chondroprotective effect on normal chondrocytes since they were efficient in reducing hypertrophy and dedifferentiation of articular chondrocytes. This effect was at least partly due to the induction of HGF secretion confirming the interest of using ASCs in therapies of osteo-articular diseases. IN progressing work, other putative factors have been identified by masspectrometry and the role of these factors is still under investigation.

With OA chondrocytes or synoviocytes, we demonstrated that ASC exerted significant anti-inflammatory effects by decreasing IL6, CXCL8/IL8, CXCL1/GROα, CCL2/MCP-1, CCL3/MIP1α, CCL5/RANTES release. In fact, these anti-inflammatory effects were evident only when ASC were co-cultured with high-inflammatory-producing chondrocytes or synoviocytes but no effect was observed in low-producing cells. Using complementary approaches, we demonstrated that basal inflammation of chondrocytes or synoviocytes in OA is crucial for priming ASC to exert their therapeutic effects. Furthermore, we gathered data demonstrating the role of COX2 and the associated production of PGE2 in these anti-inflammatory effects. We finally established that ASC-conditioned medium (ASC-CM) only partially reverts OA chondrocytes or synoviocytes inflammatory factors thus suggesting that the effects of ASC cannot be resumed to the paracrine function at a time point and that the permanent interactions between ASC and their cell partners are fundamental for counteracting the expression of different
factors crucial in OA evolution. We also confirmed that PGE2 has a role in exerting partial anti-inflammatory effects of ASC-CM on chondrocytes.

In parallel of these experiments on dedicated targets, a pan-proteomic approach was undertaken to identify new molecules putatively involved in the paracrine effects of ASC (partners 3, 11, 12). The proteomic analysis of the secretome presents some difficulties, mainly linked to the low concentration of the secreted proteins diluted in the cell culture medium, and to the presence in this medium of highly abundant exogenous proteins, deriving from serum routinely used for the culture. For these reasons, secretome is defined in serum free medium although such conditions are quite artificial and correspond to drastic conditions for cells. So, we used different strategies including a special cell culture protocol based on the SILAC approach, using a medium containing isotopically labeled amino acids, in order to metabolically label ASCs proteins, and accurately distinguish secreted proteins (labeled with heavy amino acids) from exogenous proteins (containing only light amino acids). Finally, label-free quantitative analysis of the secreted proteins from the four patients was performed based on MS peptide signal intensity using the MFPaQ software. This strategy allowed us to characterize 300 bona-fide secreted proteins in ASCs culture medium. Among them, 150 proteins were confidently identified in all four analyzed samples, of which 80% were predicted to be secreted. When all techniques are compared a restricted list could be defined and corresponds to the main proteins systematically detected in the secretome of ASC cultured according to GMP process and whatever the techniques. Among the proteins, it is noteworthy that most of them correspond to extra-cellular matrix proteins roles of which on ASC features are poorly investigated. Using SiRNA strategy, we revealed that some extracellular matrix (ECM) proteins displayed strong impact on the profile of gene expression particularly for HGF that is involved in ASC/chondrocyte interactions (see other reports) and for the inflammatory cytokines (IL1, IL6, VEGF) and immunomodulary molecules (IDO, HLA-G, Galectin...). This data clearly demonstrated that ECM could control the paracrine activity of ASC and particularly its immunomodulary activity.

Concerning the paracrine activity of ASC, Procore in collaboration (partner 11 & 12) set-up a kit to assess the secretion of hyaluronic acid by ASC from both healthy and OA donors using Procore’s HyQuant™ hyaluronic acid kit.

The following task was to identify and to isolate putative stem cells including side population (SP) cells from cartilage (partner 10). Such cells have been demonstrated to display stem cell features and could be involved in a putative regeneration of the cartilage. SP population is obtained by Hoechst 33342 dye exclusion and ABC transporter ABCG2 is responsible for this efflux. Therefore we focused on ABCG2 measurement with stained anti-ABCG2 antibodies in isolated chondrocytes. From more than 20 cartilage samples analysed by flow cytometry (cytometer/sorter FACSAria), ABCG2 expression was found only on freshly isolated cells and 1 d. after isolation, mainly on cells that did not adhere before measurement. Immunohystochemical staining identified small clusters of ABCG2-positive chondrocytes in sections of OA cartilage. Several other markers, attributed to stem/progenitor cells were also determined on the cells isolated from cartilage: besides ABCG2, we identified many traditional MSC surface markers (excluding novel marker CD200), Mesenchymal Stem Cell Antigen 1 and Aldehyde Dehydrogenase type 1 (ALDH), (evaluated by ALDEFLUOR® technique, StemCell Technologies, Canada, measured and sorted with FACSAria). Double staining for surface markers and ALDH activity showed that CD54, CD55, and CD73 were strongly enriched in ALDH-expressing cells. CD54 was recently demonstrated to have some correlation with chondrogenic phenotype, CD73 is MSC marker and CD55 or Complement decay-accelerating factor (DAF) prevents the formation of the membrane attack complex on the cell surface, thus protecting the cells from complement-mediated lysis and making these cells more viable. Gene expression analysis (Quantitative PCR, Taqman primers and probes) of ALDH-sorted chondrocytes revealed that cells expressing stem cells and differentiation genes were enriched in ALDH+ fraction.

We conclude that the studied markers identify progenitor cells with a potential of further differentiation to particular cell lineage in the cartilage. ALDH expressing cells were associated with chondrogenic potential, while their osteogenic phenotype was less expressed.

In conclusion and taken altogether this work-package brought techniques (culture protocol and GMP conditions) suitable to progress in our understanding of ASC effects on OA and to ensure a better safety and control of the therapeutic uses of these cells. Key mechanisms have been described and comfort the putative uses of ASC to treat OA. Furthermore, this WP led to publish several papers (see below) in peer-reviewed international scientific journals and one patent on the paracrine activity of the ASC.
WP 2: In vivo validation of chondroprotection effects of ASC.

Partner 1: Team Institut National de la Santé et de la Recherche Médicale U844 (INSERM) Montpellier, France

With the objective to set up the clinical trial based on the injection of autologous adipose-derived mesenchymal stem cells (hASCs) to patients with knee OA, we investigated the effect of intraarticular (IA) injection of clinical grade hASCs. Indeed, while the safety of intravenous (IV) administration of MSCs has been reported in a number of clinical trials, the safety and biodistribution of MSCs after IA injection was not tested. Our objective was to assess the toxicity of clinical-grade hASCs, their survival and their pharmacokinetics/biodistribution after IA administration in the immunodeficient SCID/Bg mouse. SCID mice received IA administration of 106 human hASCs. Several tissues or organs were recovered at different time points (day 11, 28, 90 and 186) and processed for histologic assessment or real-time polymerase chain reaction (PCR) analysis. A highly sensitive assay was used to monitor the distribution of hASCs, based on the amplification of human-specific Alu sequences. Immunological or histological analyses were performed at euthanasia. Absence of toxicity was observed after hASC injection. Alu PCR assay revealed a high sensitivity (1 hASC/105 murine cells). hASCs were detected in the joint of 90-100% of mice for the first 3 months and still observed in 60% of the mice after 6 months. With regards to the percentage of cells detected in organs, 15% of the initially injected hASCs were found at day 11 and 28 while 1.5% was still detected after 6 months. As expected, the highest numbers of human cells were recovered at the site of injection; approximately 13% of the injected ASCs stayed in the joints during the first month and 1.3% were still detected at month 3 and 6. To firmly conclude that quantification of alu sequences can be attributed to the detection of hASCs and not to DNA fragments that could be internalized by endogenous cells, we performed histological analysis. In the intra-articular space of the joint, we detected high number of cells that were mainly localized in the synovial recessi, on the edges of articular cartilage or at its surface at all the time points tested. Using fluorescent in situ hybridization these cells stained positive for human nuclei. By comparison, IV injection of hASCs resulted in rapid clearance of cells, which were detected in only 5 and 4/12 mice at day 11 and 28, respectively. In terms of cell number, 98.5% of the hASCs disappeared before day 11 and the remaining cells were mostly observed in the lung and the gastrointestinal tract and 99.9% by day 28. Importantly, absence of renal or hepatic toxicity was observed after hASC IV injection as shown by similar levels of 11 biochemical parameters in the plasma of naïve or injected mice. Histopathologic examination of the selected organs did not reveal any hASC-related findings or any hASC-related lesions or neoplasia neither on day 14 or 90 days. In conclusion, our study reports the safety and long-term persistence of clinical-grade hASCs after IA injection. We also investigated the therapeutic potential and persistence of hASCs in immunocompetent C57BL/6 mice with OA as compared to healthy mice. C57BL/6 mice developing collagenase-induced osteoarthritis (CIOA) were treated by 2.5x105 hASC in the knee joints (IA). In healthy C57BL/6 immunocompetent mice at day 1, 60.5% of hASCs were quantified in the joints after IA injection. By day 10, 8% of hASCs were detected in 30% of mice after IA injection but no signal was present at a later time point. Local injection of hASCs showed therapeutic efficacy for at least 35 days in the CIOA model. The percentage and distribution of hASCs were however similar in arthrosic and healthy mice, independently of their inflammatory status (manuscript in preparation). Altogether, these data support the safety of using IA delivery of hASCs in the treatment of rheumatic diseases. The degree of inflammation did not affect the persistence of hASCs.

Partner 4: Team Istituto Ortopedico Rizzoli, Bologna, Italy.

The preclinical study was aimed to evaluate the therapeutic potential of an intra-articular injection of autologous Adipose-derived mesenchymal Stem Cells (ASC) and the optimal cell dose for osteoarthritis (OA) management in an OA rabbit model. In particular, we investigated the local biodistribution of ASC in rabbit knee joints and their action on cartilage, synovium, meniscus and bone. The biodistribution studies gave evidence of ASC homing in the meniscus and lining layer of the synovial membrane, in close proximity to macrophages that would suggest a trophic activity. Between two different cell doses employed in the study, the lowest one showed strong contribution in promoting cartilage repair by counteracting cartilage OA progression through: i) the reduction of catabolic and inflammatory markers in menisci and synovial membrane evaluated by histological and immunohistochemical assays; ii) the down-regulation of catabolic processes at subchondral bone level.
observed by micro-CT analysis. In particular, ASC treatment reduced matrix degrading enzymes like MMP-1 and MMP-3 and inflammatory cells in synovium and, cluster formation in menisci. This led to cartilage repair, reporting a well-organized tissue with a low Laverty’s score mediated by: i) up-regulation of anabolic markers; ii) down-regulation of hypertrophic and fibrotic markers; iii) down-regulation of catabolic and inflammatory markers. As concern the bone component, the lowest ASC dose group displayed better results in terms of epiphyseal trabecular bone mineral density, trabecular thickness and trabecular separation (manuscript in preparation). In conclusion, the findings of this study demonstrated that an intra-articular injection of ASC exerts a chondro-protective role promoting a series of anabolic processes to allow the maintenance of a good collagen and proteoglycan network and at the same time inhibiting inflammatory and catabolic processes in synovium and menisci. Therefore, ASC therapy could represent a valid therapeutic option for OA treatment.

Partner 6: Team Friedrich-Alexander Universitaet Erlangen-Nuernberg (ERU-FAU), Germany.

Objective:
Adipose-derived stromal cells (ADSCs) express immunosuppressive characteristics, mediating anti-inflammatory and chondroprotective effects in osteoarthritis (OA). In the experimental collagenase-induced OA mouse model we wanted to determine the potentially protective role of Fra-1/AP-1 overexpressing (Fra-1tg) ADSCs in OA.

Methods:
Experimental OA was induced by intra-articular injection of collagenase into the knee joints of C57BL/6 mice. Wild-type (wt) or Fra-1tg ADSCs were isolated from inguinal fat pads of 6 week- or 8 week-old mice, cultured until passage 1 and injected at day 7 after induction of OA into the knee joints. Histologic analyses for cartilage destruction, empty chondrocyte lacunae and chondrocyte apoptosis were performed 6 weeks after OA induction. Stromal vascular fractions (SVFs) of inguinal fat pads from wt and Fra-1tg mice were analysed by fluorescence-activated cell sorting (FACS). Furthermore the adipogenic differentiation capacity, mRNAs- and cytokines-profiling of wt and Fra-1tg ADSCs were investigated.

Results:
At day 42, cartilage destruction and empty chondrocyte lacunae were significantly decreased in osteoarthritic knee joints treated with Fra-1tg ADSCs derived from 8 week-old mice, accompanied by a decrease of chondrocyte apoptosis when compared to the corresponding wt ADSC treatment. However after treatment with Fra-1tg ADSCs derived from 6 week-old mice, only a slight decrease of chondrocyte apoptosis was observed, without further protective effect in OA. In fact, SVFs derived from 8 week-old Fra-1tg fat pads revealed an increased number of adipogenic progenitors compared to wt SVFs, whereas adipogenic progenitors in SVFs derived from 6 week-old mice were similar in wt and Fra-1tg mice. However, treatment of OA-induced knee joints with pre-sorted adipogenic progenitor enriched wt ADSCs, derived from 8 week-old mice, showed no increased protective effects compared to the corresponding unsorted wt ADSCs treatment, proving that Fra-1 over-expression is necessary for the increased protective effect. Actually, Fra-1tg ADSCs showed a decreased adipogenic differentiation capacity, which is characterized by decreased mRNA-levels of C/EBPα, PPARγ2 and Glut-4. Moreover, Fra-1 transcriptionally inhibited the pro-inflammatory cytokine IL-6, accompanied by a change in STAT3-signalling in Fra-1tg ADSCs. Finally, important regulators of extra cellular matrix (ECM) homeostasis, such as periostin (POSTN), Spondin 1 (Spon1), Pentraxin 3 (PTX3) and plasminogen activator inhibitor 1 (PAI1) were also found modified at mRNA- and protein-level in Fra-1tg ADSCs.

Conclusion:
Our findings indicate that over-expression of Fra-1 in ADSCs protects from collagenase-induced OA and is mediated by decreased IL-6 secretion accompanied with modified levels of ECM homeostasis regulators, such as POSTN, Spon1, PTX3 and PAI1.

Partner 7: Team Radboud Universiteit Nijmegen (RUNMC) Nijmegen, Holland
Much evidence is accumulating that synovitis significantly contributes to joint destruction in a subpopulation of patients with osteoarthritis. Recent findings indicate that stem cells apart from tissue repair may also have anti-inflammatory effects. In the ADIPOA project, we investigated the effect of local deposition of adipose derived stem cells (ASCs) in murine OA knee joints on the development of cartilage destruction and formation of osteophytes. We compared two mouse models of OA which differ in synovitis. In one model, OA is induced by intra articular injection of collagenase (CiOA) and is characterised by smouldering low grade synovitis and eventually severe joint destruction. In the second model OA is surgically induced by dissection of the medial anterior meniscotibial ligament (DMM). The disease course of DMM is not associated with synovitis and joint destruction is somewhat milder when compared to CiOA. A single injection of ASC into mouse knee joints with early stage (day 7) collagenase-induced OA, strongly inhibited synovial thickening, formation of enthesophytes associated with ligaments and cartilage destruction as measured at day 42 after treatment using histology (1,2). A dose of 20,000 cells was thereby more effective than a five times higher dose. Interestingly no amelioration of joint destruction was measured when ASC were injected at day 14 (4) after induction of CiOA. Next, ASC treatment was performed in CiOA with high and low smouldering synovitis. Significant ameliorating effects were only found when ASC were injected in OA joints with high but not when injected in joints with low synovitis (4). This suggests that synovitis may be related to stimulation of anti-inflammatory effects of ASCs. In line with that, injection of ASC into mouse knee joints with early (day 7) or late (day 14 and/or day 21) stage surgical induced DMM in which synovitis is scant also failed to ameliorate development of joint destruction (3). We recently found that synovial macrophages and “alarmins” S100A8/A9 (important macrophage products) are crucial players in mediating cartilage destruction as well as osteophyte formation (3). Local deposition of ASCs in CiOA knee joints, home to the subintimal synovial lining layer and GFP-labeled cells were visualized in close interaction with macrophages (2). Activated synovial macrophages produce large amounts of alarmins S100A8/A9 which leak into the joint cavity and from there into the blood. We measured significantly elevated serum levels of S100A8/A9 throughout the disease course (up to day 42) of CiOA. A single injection of ASC into a day 7 CiOA knee joint caused a rapid (already at day 2 after injection) and significant down regulation of cytokines IL-1 (60%) and S100A8/A9 (50%) in synovial washouts (4). Gene analysis studies (microarray) on synovium showed that many genes (>100 genes) were downregulated already at day 2 after ASC treatment. At day 14 after ASC treatment, serum S100 levels were significantly lower (70%) when compared to non-treated OA controls (4) suggesting that these proteins might be good markers for measuring ASC efficacy.

Partner 9: Team: Research Center Magnetic-Resonance-Bavaria (MRB); Germany.

In the primary MRI report the main issue in data processing was the appearance of oedemas and effusions close the knee cartilage after the surgeries which were difficult (dGEMRIC, T1p) to almost impossible (sodium) to discriminate from the cartilage, especially at the time point 3 weeks after surgery. To allow for a better analysis of the acquired data, different modifications and adaptations in the post processing algorithms were implemented and tested and all acquired data sets were processed new. In the case of the sodium MRI, the proton MR images and relaxation time constants were used to assess the “real” borders of the cartilage semi automatically in the sodium MR images. Of course, this method gives only an estimation of the precise borders and is therefore prone to errors, but at least allows analyzing the acquired data at all. Actually, using this adapted post processing algorithm, it was now possible to determine a quantitative sodium concentration for every goat and every time point. Fig. 1a shows a significant (p = 0.005) decrease in the sodium signal of the cartilage within the first 3 weeks after the defect surgery. No significant change could be observed from there on. Therapy and control group still exhibit no significant difference at all time points.

Using individual subregions of the cartilage and limiting the data analysis to the weight bearing zone allowed to determine small changes in the dGEMRIC index dG1 which can be attributed to OA without losing this information by averaging over the still healthy bulk cartilage. Since the dGEMRIC indices dG1 and dG2 are highly correlated the following results are only shown for dG1. While dG1 still shows no significant difference between the therapy and control group at all-time points, by dismissing a spike in the therapy group, both groups show now a significant decrease from the beginning to the last time point (cf. Fig.1b). Limiting the analysis to the weight bearing zone did not lead to a significant difference between both groups in their T1p values at any point of time. But, the therapy group yields a significant increase in T1p during the first three weeks and a
significant decrease from week 3 to week 16 (Fig. 1c). In order to verify the different MRI methods and to compare their individual results, a correlation analysis was performed between all MRI methods: dGEMRIC indices dG1, dG2, T1ρ and sodium MRI. Correlation of the MRI results and histology results is still not possible because not all histology results are available yet. Non surprisingly, a very strong correlation (**) was observed between dG1 & dG2 which differ only in considering or not considering the T1 measurement before contrast agent administration. Moreover, there are moderate correlations (*) between T1ρ & dG1, T1ρ & dG2 and Sodium & dG1. (cf. Fig.2 & Tab.1).

In summary, the different MRI methods visualize the on-going OA. Since T1ρ is sensitivity to early changes in the cartilage, only T1ρ shows first the on-going OA, but also a significant change in the therapy group at the end which can indicate the initiation of healing processes. The correlations between the different methods, match the underlying biological mechanisms which the methods are sensitive to: sodium MRI reflects the proteoglycan and GAG concentration, dGEMRIC is sensitive to the proteoglycans and mainly to the GAG content, while T1ρ yields information mainly about the proteoglycans and collagen.

WP 3: Safety, Security and regulatory issues

The main objective of Work-package 3 within the consortium was to provide autologous adipose stromal cells (ASC) under good manufacturing practices (GMP) conditions for clinical trial in osteoarthritis (OA) therapy.

The medicinal product was defined as autologous adipose stromal cells (ASC) from the abdominal subcutaneous fat (liposuction). After extraction, autologous cells are expanded for 14 days and cells are injected in the knee of osteoarthritis patient.

This work consisted to determine:
- The safety of cell injection in animal model
- Stability studies for optimal storage and transport conditions
- obtain approval from French and German regulatory agencies for clinical trial
- Provide GMP grade cells for the clinical trial in France and Germany.

Evaluation of the medicinal product’s safety

Different analyses were performed to evaluate the potential susceptibility of in vitro expanded ASC for genetic alterations. Cell expansion from healthy donors has been compared to ASC from OA patients. Cells were cultured during successive passages to study long-term evolution of proliferation potential. As expected, we observed a progressive decrease of proliferation until senescence at around passage 15 (about 5 months in culture). The proliferation of ASC from OA patients was very similar for all donors and the cells stop to proliferate (replicative senescence) between 30 and 60 population doublings. We did not observe a restart of the proliferation and transformation at senescence.

Karyotype analysis revealed no abnormalities for ASC (from liposuction) at earlier passages. During the long-term culture, transient karyotypes abnormalities has been observed but all culture ended at senescence. DNA damage accumulation in vitro by microsatellite instability (MSI) analysis has been evaluated. No cases of microsatellite instability were observed and allele patterns were maintained overall the culture period for all the analysed donors, indicating that repeated duplications in vitro did not alter genetic stability of short repeated sequences.

To complete the genetic analysis, the mRNA of genes involved in the transformation process (c-myc, p53, hTert) has been evaluated. The genes did not differ between ASC with or without karyotypic abnormalities. The mRNA of c-myc or p53 remained stable and hTert was not expressed.

To evaluate the safety of ASC in vivo, animal model (SCID mice) studies were conducted for tumorigenicity. SCID mice (males and females) received 2.106 human ASC by intravenous injection. Mice were divided in 2 groups and sacrificed 2 weeks or 3 months after injection. During experimentation, no obvious clinical signs of stress or behavioural modifications were observed at 14 days or 90 days after injection compare to control group. The food consumption was recorded every week and was not
different whatever the group.

At day 14, haematological analyses were performed to measure a possible short answer of the mouse immune system to the systemic injection of ASCs. We didn't obtain any difference between ASC-treated groups and control groups for red blood cell count, haemoglobin level, haematocrit, platelet count and plateletcrit.

At day 90, biochemical analyses were performed to detect a possible toxic effect on the long-term in the mice receiving a systemic injection of ASCs. The most important effect of ASC observed 3 months after cell administration was a decrease in triglyceride levels which cannot be associated with pathologies. No significant difference between groups was observed for total bilirubin, cholesterol and glucose concentration. The data reflect the absence of any significant pathological biochemical toxicity.

Histopathologic examination of selected organs did not reveal any tumour after ASC injection in mice killed on days 14 and 90.

To evaluate biodistribution of ASC, SCID mice received 1.106 of cells by intra-articular injection. Mice have been divided in 4 groups and sacrificed at day 11, day 29, day 90 and day 187 or 206.

During the 6 months of experimentation, no obvious clinical signs of stress or behavioural modifications were observed. After 11 and 29 days, most of the injected human ASC were detected at the site of implantation: 12844 ± 5823 cells in the joints in 9/10 mice at day 11 and 15534 ± 5760 cells in 9/9 mice at d29. The number of detected cells corresponds to 1 to 3% of the total number of injected cells. Very few cells were detected in the other organs at day 11 and day 29 and in only some individuals. Main targeted organs were kidney, heart and brain in 4 to 6/10 mice at day 11 and lung and brain in 3/9 animals at day 29. After 3 and 6 months, similar results were obtained. Presence of ASC at the site of implantation was always observed in almost all animals treated. At day 90, ASC were detected in large numbers in the muscle of 3 mice and in the brain of one mouse. Importantly, at these later time points, detection of ASC was recorded in fewer organs and in few animals. This biodistribution study revealed that at the different time points after the intra-articular injection of ASC, the presence of the cells was detected in almost all injected joints. However, part of the injected cells probably died rapidly since after 11 days, only 1% of the ASC remains in the joint and this number was constant throughout the experiment till day 90 with a tendency to decrease at 6 months. Part of the injected cells is likely to enter the bloodstream and home at different locations in the body and in particular, the brain and the adjacent muscle but in very low amounts and in only few animals. Of importance, the majority of the cells were detected at the site of injection where they remain for at least 6 months.

Definition of optimal transport conditions

In the clinical context, cells had to be transported from the production centre to the patient in the clinical site in France (Montpellier) and in Germany (Würzburg). To avoid any manipulation of the medicinal product in clinical sites, we had chosen to deliver cells in suspension in transport/injection medium. To reach this objective, a stability study has been conducted in two storage/transport liquid solution at +4°C/+8°C: human albumin 4% with or without 10% of polyionic solution containing glucose.

Different parameters (numeration, viability, phenotype, CFU-F frequency, cell proliferation, apoptosis and differentiation capacities) were studied at five different concentrations (0.4, 2, 2.5, 5, and 10 million cells/mL) during a storage period of 48 hours. ASC obtained from GMP production has been centrifuged and re-suspended in preservation solution at the appropriate concentration. Cell suspension was packaged in syringe and store at +4°C/+8°C. To assess the efficiency of the 2 preservation solutions, the evolution of viability and total cell concentration has been measured at regular time during 48 hours.

Results revealed that the better preservation solution for cells in liquid solution at +4°C/+8°C was human albumin (3, 6%). and 0, 5 % glucose. In these conditions, cells can be stored in syringes at least 28h whatever the concentration. Others parameters have been tested after 28 hours of storage. Analysis of apoptosis revealed that cell death were less important in presence of glucose and do not exceed 8 % after 28 hours of storage. Cell surface markers were not affected and osteogenic, adipogenic and chondrogenic capacities was maintained. Cell expansion capacities were slightly lower and progenitor content was reduced after storage but this difference was not found again after a further cell expansion procedure.

These experiments confirmed that the better preservation liquid solution for the medicinal product transport was human
albumin containing glucose during 28 hours.

Regulation agencies approval

In order to prepare the phase I clinical trial submission, regulatory authorities have been met in Germany (PEI) during March 2010 for a scientific advice and in France (ANSM) during September 2010 for an informal meeting. The regulatory dossier was set-up in the first trimester of 2011 with safety data and stability studies and has been submitted in August 2011 in both countries. This dossier consists of 3 main parts: the clinical protocol, the investigator brochure and the investigational medicinal product dossier.

Following the initial submission, French and German agencies asked for complementary information and requests respectively in October 2011 and December 2011. All points have been argued and additional information has been provided for France in November 2011 and for Germany in March 2012. Finally, the clinical trial has been authorized in December 2011 in France and in March 2012 in Germany.

As the complementary information and request was different in the 2 countries, a new version of the protocol has been issued including requests from French and German agencies for harmonization but also for corrective actions regarding first patients’ inclusion. These substantial modifications have been submitted to regulatory agencies and accepted in January 2013 and February 2013, respectively Germany and France.

GMP grade production for clinical trial

The active substance has been manufactured from the patient’s adipose tissue, which had been transported from the clinical site to the manufacturer facilities in Toulouse (France). The manufacturing process for expanded ASC consists on enzymatic digestion of adipose tissue and cell expansion for 14 days. Medicinal product has been manufactured under GMP conditions using aseptic procedures and disposable sterile single-use supplies for all product contact steps; therefore no product directly contacts equipment. All the cell culture has been performed according to cell engineering unit quality system, with established standard operating procedure and operating methods. All culture disposables were EC marked and all reagents were GMP-used.

The culture process was designed to select and amplify the ASC already present in the adipose tissue. The culture times were optimized both to allow the selection of ASC for 8 days (primary culture) and the amplification of cell population for 6 days (first passage). The culturing times were reduced as much as possible to limit the waiting time for the patient between the biopsy and the injection of the cell finished product. Several controls have been implemented to ensure quality and safety of the cells during the manufacturing process. All quality controls, including batch release controls, have been defined, validated and approved by regulatory agencies.

The design of clinical study was to investigate the clinical safety of intra-articular ASC in patients with osteoarthritis of the knee joint and indication for arthroplasty. Another objective was to evaluate tolerability of 3 different dosages of cells administrated in a single injection. For this reason 18 patients have been enrolled in 3 cohorts receiving 2.106, 10.106 or 50.106 cells in a volume of 5 mL.

GMP production of cells has been performed and injected for 18 patients between March 2012 and December 2013. For the 1st cohort (2.106 cells), a safety period of 3 months has been observed before enrolled the 5 others patients. Two others safety period of 30 days has been observed between cohort to evaluate adverse effect. For all patients, the cells dose has been reach. Quality controls for release were conformed to specifications including sterility test, mycoplasma test, endotoxin test, hTERT expression (qRT-PCR) and Oct-4 expression (qRT-PCR).

No adverse effect has been observed after injection for all patients.

WP 4: Clinical trial endogenous ASC injected intraarticular in OA.
Introduction
The aim of the ADIPOA project was to develop a new cell based strategy for patients suffering on knee osteoarthritis (OA). Up to now, no disease modifying treatment exists to obtain a sustainable improvement in joint function, beside total knee arthroplasty. The new treatment strategy bases on the intraarticular injection of autologous, adipose derived stromal cells (ASCs). ASCs have the ability to secrete growth factors, to stimulate cells in the knee joint, and to influence the inflammatory environment.

Study design
Our initial study design included patient with moderate OA. Scientific advises given by the German and French competent authorities (PEI, ANSF) addressed the safety aspects of our trial with the recommendations:
- To include only patients with need of total knee arthroplasty
- To follow-up patients for 12 months after intra articular ASCs injection
- To include second only after first has reached end of study (TKA)
- To include a safety period of four weeks between the next escalating doses group

In 2011 the final design was submitted to Competent Authorities by partner 8 (WU) and partner 1 (CHRU MTP).

- Protocol Title:
  A phase I, prospective, bi-centric, single-arm, open-label, dose-escalating clinical trial to evaluate the safety of a single injection of autologous adipose derived mesenchymal stromal cells in the treatment of moderate or severe osteoarthritis of the knee joint
- Sponsor:
  CHRU Montpellier, Department research and innovation
  191, avenue du Doyen Gaston Giraud - F-34295
  Montpellier cedex 5 - FRANCE
- EUDRACT number: 2011-000183-10
- Short title: ADIPOA 01
- Number of centres: 2
- Phase: Phase I/II
- Coordinating Investigator(s): Pr Christian Jorgensen / Dr. med. Lothar Seefried
- Objective:
  Primary: To study the safety of a single injection of autologous adipose derived mesenchymal stromal cells (ASCs) on patients with moderate or severe osteoarthritis of the knee (OA).
  Secondary:
  To study the efficacy of a single injection of autologous adipose derived mesenchymal stromal cells on patients with moderate or severe osteoarthritis of the knee (OA).
- Primary Endpoint: Safety and tolerability of a single intra-articular injection of ASCs in ascending dosages. Safety and tolerability will be evaluated by recording adverse events (AEs) and serious AEs (SAEs) throughout the study.
- Study Design: bicentric, open-label, prospective
- Randomization: There will be no randomization procedure in this study; patients will be recruited sequentially into cohorts 1 to 3, with escalating dosages.
- Investigational Medicinal Product: Autologous adipose derived stem cells administrated for intra-articular use.
- Treatment: Escalating single dose:
  Group 1 : 2 x 10^6 ASC intra-articular injection (5 ml)
  Group 2 : 10 x 10^6 ASC intra-articular injection (5 ml)
  Group 3 : 50 x 10^6 ASC intra-articular injection (5 ml)
- Study Population: Patients with symptomatic moderate or severe osteoarthritis of the knee and indication for total knee arthroplasty.
Visit schedule: Each patient will attend a total of 13 scheduled visits, which will be completed over a period of 13 months from screening visit to end of study.

Number of patients: 18 patients. 3 groups of 6 patients will sequentially be included

Study duration: Each patient will receive one single administration of the cells and will be followed for 12 months. Each patient will be follow-up during four years with routinely examinations for safety issues. The study is planned to begin at the end of 2011. Clinical trial application is expected in September 2011.

Follow-up measures: Safety care outside the study protocol are sequentially visits up to four years after the final visit (visit 13)

Key Inclusion Criteria: Male and female, 50 to 75 years old. OA diagnosis should fulfill the criteria of the American College of Rheumatology (ACR) with moderate or severe medial and/or external femorotibial knee osteoarthritis (OA) (stage 3 or 4) and indication total knee arthroplasty.

Key Exclusion Criteria: Any disease or medication affecting the bone or cartilage metabolism, including corticoids.

Secondary & Key Exploratory Efficacy Endpoints:

Efficacy will be assessed by measuring:
- functional status of the knee
- pain-specific assessment
- WOMAC (Western Ontario and McMaster Universities osteoarthritis index)
- global patient assessment (visual analog scale, Short-Form 8)
- Short Arthritis assessment Scale (SAS)
- range of motion of the target knee joint,
- quality of life
- decrease in rescue paracetamol medication
- imaging through MRI and scanner evaluation

Methodology/criteria for evaluation:

Primary Safety Variables:
Type and incidence of AEs and serious AEs (SAEs) during the treatment period. AEs will be monitored throughout the study and recorded and coded according to medical dictionary for regulatory activities (MedDRA) dictionary criteria.

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

Statistical Methods: Adverse events will be described with respect to seriousness, intensity, relationship to treatment, action taken and outcome of the adverse event. Response to treatment will be presented using descriptive statistics.

With respect to this first-in-human clinical trial safety was addressed by various precautions:
- An independent Data and Safety Monitoring Board (DSMB) was established.
- Each subject received a single dose of the ASCs only.
- A complete follow-up period of 8 weeks for the first subject was elapsed, before the subsequent subjects of the same cohort was injected with ASCs.
- Treatment of subsequent subjects was allowed only, if no serious adverse reactions have occurred in the first subject of the respective dosing cohort.
- A time interval of 4 weeks was respected between injection of the CPMP into the last subject of a dosing cohort and of the first subsequent of the next dosing cohort.

All other safety parameters assessed in the ADIPOA trial was recorded and evaluated for supportive safety purposes.
- Physical examination
- Knee examination (e.g. swelling, effusion, and signs of infection)
- Clinical global assessment
All patients have been enrolled by October, 31th, 2013 (last-patient-in) and final visit will take place in December, 2014 (last-patient-out). Harvesting of the adipose tissue and intraarticular injection was performed in the operation theatre. Transport and manufacturing of the ASCs respected rules of good manufacturing practice (GMP).

Results

Refusing of TKA
Competent authorities advised to include only end stage OA in order to fulfil the safety aspects of this first-in-human clinical trial. Therefore, total knee joint replacement (TKA) has been scheduled three month after ASC injection. Up to now, only two patients received TKA within the 1-year trial duration. Due to the improvement of pain and mobility, the majority of patients refused TKA.

Adverse events (AE) and serious adverse events (SAE)
No serious adverse events related to intraarticular injection of ASCs was observed. Adverse events property related to ASCs injection was documented in the high doses group. Two patients reported reddening and swelling of the knee joint after injection of the high doses ASCs for a few days.

Pain Assessment
Pain serves as a safety endpoint and efficacy parameter. Pain was assessed using the Visual Analog Score (VAS). Significant differences in pain reduction are present between “before injection” and 12 weeks and ½ year results over all dose groups. Comparing the tree dosage groups among each other, no differences were found.

WOMAC
The Western Ontario and McMaster Universities Arthritis Index determines the overall level of disability by assessing pain, joint stiffness, physical as well as social and emotional function. A significant improvement of pain and activity (lower values, Fig. 4) can be shown for all three dosage groups until 6 month after ASC injection. Comparing the tree dosage groups among each other, no differences were found.

Improvement of pain and WOMAC score after intraarticular injection of ASCs
Data of each dose group were analysed by a > 20% improvement of the pain score (VAS) and WOMAC score. Pain scoring and WOMAC questionnaire together with further data on patients outcome give evidence that IA administration of ASC is a save therapy and lead to an improvement of joint function and pain.

The final design of the ADIPOA trial was accepted by French Competent Authorities in December, 2011, and by German Competent Authorities in March 2012.

Potential Impact:

a. Impact
The Alliance for Regenerative Medicine (ARM) estimated that over 700 companies with regenerative medicine focus are in existence. These include divisions of multinational corporations to smaller sector-driven organisations. It is currently estimated by ARM that over 1900 clinical trials relating to cell based therapy engaged globally. This vast number of trials is represented by over 250 companies assessing cell based therapies in a wide variety of diseases. Currently, there are over 40 cell therapy products available, with musculoskeletal products representing 35%. The recent ‘Translational Regenerative Medicine: World
market prospects 2014-2024' report indicates that the global regenerative medicine market is now estimated at 6.4 billion USD. Assuming that strong efficacy outcomes continue to emerge from clinical testing, it is estimated that the numbers of patients who will seek stem cell therapy in Europe alone will be at least 600,000 per year. Nearly 50 companies around the world are particularly active in the field of regenerative medicine relative to cartilage and osteoarthritis. Of these companies, products from Genzyme (Carticel, MACI), Medipost (Cartistem) and TiGenix (ChondroCelect) are classed as the leading commercial cell therapies globally. ChondroCelect® (TiGenix) was the first cell based therapy to reach commercialisation subsequent to approval in 2009 by the European Medicines Agency. The autologous chondrocyte implantation technique is commercially available in Belgium, the Netherlands, Luxembourg, Germany, UK, Finland and Spain. More recently in 2012, Medipost Biotechnology has received Korean FDA approval for the manufacture and sale of their allogenic umbilical cord blood product CartiStem® for the treatment of traumatic and degenerative OA. Medipost are currently recruiting for phase I/II clinical trials assessing the safety and efficacy of CartiStem® (NCT01733186) and a phase III trial assessing efficacy versus microfracture (NCT01626677) to obtain US FDA approval. In Australia, Regenus report improvement in sleep quality, pain, function and reduced need for additional medication in 80% of the 335 patients subsequent to injection of HiQCell (autologous ASCs).

Other products are also being tested in clinical trials. Zimmer Orthobiologics particulated juvenile cartilage implant DeNovo® NT graft received is a FDA-listed tissue product since 2007 and has been used in over 3000 patients. This product is currently being tested in a phase 4 trial (NCT01670617). Histogenics is also engaged in a phase III study of their NeoCart® product (autologous chondrocyte embedded in collagen scaffold) compared to microfracture (NCT01066702). Cartilage Autograft Implantation System (CAIS, DePuy-Mitek) is also involved with phase III trials. Others such as Osiris Therapeutics (Chondrogen®: Intra-articular injection of MSC in HA; NCT00225095), ProChon Biotech (BioCart®: scaffold containing autologous chondrocytes; NCT00729716) and Mesoblast (Allogenic mesenchymal precursor cells in Rheumatoid Arthritis; NCT01851070) are engaged in phase II trials. ChondroCelect® and other regenerative treatments have a price between €14000-20000 per patient. Several of the marketed products such as ChondroCelect are also not reimbursed. We have planned a cost around €10,000 per patient which is substantially lower. While several regenerative products exist within the European market these tend to involve either a scaffold based system with/without cells i.e. Chondro-Gide (Geistlich) or autologous chondrocytes as a cell-source. There are particular disadvantages with both these points. The majority of MSC-based therapies are now adopting a streamlined scaffold-free approach given that delivery and regulatory approval are significantly easier from a strategic view point. Additionally, the harvest of autologous chondrocytes is significantly less desirable compared to the ease of harvest of MSCs, particularly ASCs. Harvest of chondrocytes involves issues with donor site pain and morbidity. With a potential market of over 70 million OA sufferers within Europe, we estimate that approximately 300-1000 patients within the EU will be treated annually during the first three years subsequent to launch of the of the ADIPOA product. The production of the cells will be granted to EFS, NUIG and UULM, which have GMP facilities and will work as contract manufacturing organizations. These centres will be a leading force in commercialization and the cell product development.

This proposal outlines a cell based therapy that will be the first of its kind within Europe. Consequently, this will place the EU in a position of leadership within regenerative medicine. Consequently, this proposal will pave the way for improving safety and efficacy procedures significantly. The added value will be provided not only by inputs from the academic participants, but also by industrial background associated with SMEs. Part of this strategy involves the development and validation of a patented, personalised, whole-blood assay to quantify the anti-inflammatory potency of cultured stem cells for commercialisation. Commercialisation of the technology will lead to facilities with highly trained staff in hospitals across Europe with potential for global expansion.

b. Results of in-patient regenerative medicine research

OA, a prevalent chronic condition with a striking impact on quality of life, represents an enormous societal burden that increases greatly as population’s age. The ADIPOA phase IIb multicentre clinical trial will assess the efficacy of a single intraarticular injection of autologous ASCs in treating mild to moderate knee OA. Two different doses of ASCs will be tested (50 patients each), with outcomes evaluated based on a pain subscore (WOMAC) at 12 and 24 months, by comparison to a control group receiving HA. Potential structural benefits and effects on disability and quality of life will also be measured (WP3).
builds on a previous first-in-man study (ADIPOA1), which proved the feasibility and safety of autologous ASC therapy and suggested the most effective dose, but did not have the power to demonstrate statistical significance of the treatment. Therefore:

1. This is the first European clinical trial with the potential to reach significance in assessing ASCs for the treatment of OA, and demonstrates in-patient the efficacy and repeatability of this treatment.
2. The network of participants, formed of teams studying the biology of ASCs, SMEs developing novel devices and research-oriented clinicians, will provide new and efficient procedures for the translation to an innovative OA therapy.
3. The translational process into the clinical application is based upon and augmented by a strong partnership with SMEs and regulatory bodies.
4. Communication with regulators through ECRIN will set the standard for efficient harmonisation of trans-European cell therapy trials.
5. ADIPOA will also address gaps in our understanding of the functional role of ASCs and furthermore, will establish general tenets of mesenchymal stem cell anti-inflammatory action, and therefore will have applications beyond cartilage aspects, paving the way for complex tissue regeneration.

Thus, ADIPOA constitute a major step forward in the translation of research findings into the clinic, allowing statistical determination of the clinical potential of ASCs in the treatment of OA and forming the foundation for the development of this and related emerging regenerative therapies. Ground work will be in place for pivotal phase III testing with potential to attract industrial backing and realise the prospect of improved health outcomes, and more active and healthy ageing.

c. Growth and competitiveness of sector

In the global research and development effort in stem cell therapy that has arisen over the past decade this study is unique in that it will take the field far beyond ideas and very close to marketability and delivery: It will take ADIPOA1 to a point of realizable value for patients, clinicians and industry.

6. ADIPOA will strengthen and extend the network of European expertise established by ADIPOA1, including to multiple clinical trial centres. The ADIPOA consortium comprises 18 participants from 6 countries, i.e. several hundred researchers from public agencies, universities and SMEs, as well as clinicians.

7. By definitively testing ASCs in the treatment of OA patients and providing unambiguous evidence of a therapeutically useful outcome, this study will stimulate investment by large pharma and medical device companies in stem cell therapy, which has to date been limited. This will also foster more widespread acceptance of stem cell therapy within the healthcare professions.

8. Through strong partnerships with SMEs, e.g. Human Med AG and ARIEL Srl, ADIPOA should stimulate economic growth and competitiveness, as well as having a social impact, in the EU. The advances that will be made by this project should enable collaborating SMEs to compete internationally and expand beyond Europe, establishing wider markets and more business opportunities.

9. ADIPOA will further protect novel findings and advance commercialisation of ASC and other MSC therapies by development of the quantification of the anti-inflammatory potency of therapeutic stem cell products for individual patients. Without such a larger study, the field of cellular therapy will continue its slow pace, lacking the validated clinical data needed to attract serious attention by investors and industry.

ADIPOA has brought together world leaders in the biology and translation of MSC therapies for OA with seminal work in the area. Implementation of the successful ADIPOA Phase I clinical trial cemented the reputation of the group in this space and benchmarked Europe as a major player and leader in the area of novel regenerative therapies for OA. The ADIPOA project will take advantage of a high level of communication and scientific exchange between the different EU partners and underscore this European leadership position in cellular therapies not only for treatment of OA but many other incurable diseases with an inflammatory component. The project will catapult Europe to the fore as a location to translate and commercialise cellular therapies by

• Demonstrating effective cross-sectoral European collaboration in regenerative medicine research. This collaborative environment will be reflected at all phases of the proposed project with harmonisation of cell production, regulatory management, design and implementation of the phase IIb clinical trial, research into the mode of action and potency of ASCs, and project management.
• Contributing to the European patent portfolio and industrial landscape by automation and development of closed system production methodologies for ASC systems with SME partners. Furthermore, ADIPOA will test a patented European technology for the rapid, personalised prediction of patient response to cellular-based anti-inflammatory therapies.
• Resolving issues of quality assessment, technical and legal harmonisation between different EU member states and thus creating an attractive backdrop for pivotal demonstration of cellular therapies in Phase III trials and ultimate translation of the novel therapies to clinical practice.
• Disseminating results to increase awareness and acceptance of new therapies by the European populace and establish ease of selection of multinational cohorts of patients for pivotal clinical trial implementation. ADIPOA will thus place the EU at the forefront of regenerative medicine initiatives.

d. Innovation capacity & knowledge integration
OA is responsible for substantial pain and functional disability. Current treatments for OA largely focus on pain management and the eventual replacement of the affected knee or hip joint. The usually recommended treatment includes physical therapy, analgesics, local infiltration of corticosteroids and HA, without structural benefit. It is widely accepted that mesenchymal stem/stromal cells derived from a number of sources including adipose tissue have the capacity to protect tissue from degeneration and to support a reparative process through their paracrine activity. As such 10. Autologous ASC therapy is potentially an accessible and effective treatment that will reduce the burden of this disease.
11. The ADIPOA consortium has shown in a 2-centre Phase I study that intraarticular injection of a single dose of autologous ASC to the knee was well-tolerated, gave rise to no adverse effects, and resulted in an improvement in pain score and functional outcome. Additional data generated in preclinical studies supported this finding.
12. The ADIPOA Phase IIb multicentre clinical trial will be the first clinical trial to reach significance in assessing ASC for the treatment of OA and will assess the efficacy and repeatability of this treatment in patients.
13. This will be a crucial milestone in the treatment of a condition which has evaded all previous therapeutic efforts other than joint replacement surgery.
14. More broadly, stem cells are ideal candidates for the treatment of a wide variety of inflammatory and degenerative diseases, such as cardiac indications and diabetic wounds.
15. Thus, MSC therapy could potentially provide an alternative option in a range of conditions when no curative treatment is available or for patients who are refractory to current treatments.
The global market for regenerative medicine applied to OA is estimated at €130 million, and the European market at €60 million (World Stem Cells 2009, BCC research). This is an emerging market and these numbers are set to increase exponentially.
• Although cell engineering is already developed in many of the EU member states where clinical protocols using mature chondrocytes for implantation in femoral condyle defects are being pursued, the number of currently available treatments is low and many developments are taking place.
• Globally, there are approximately 50 companies that are particularly active in the field of regenerative medicine applied to cartilage and OA. As described above, 10 of the companies have products on the market. In reality, only one product addresses the therapeutic indication of OA (ChondroCelect® - TiGenix); other products target cartilage defects but can be used on patients with OA. There are 4 products currently in Phase III: Denovo®, Neocart® and CAIS®, Medipost®. 3 products are currently in Phase II: Replicart® (Mesoblast), Chondrogen® (Osiris), Biocart® (Prochon).
• ADIPOA will focus on cell therapy for OA and will place the EU at the forefront of this field, via a Phase IIb multicentre clinical trial that will assess the efficacy of a single intraarticular injection of autologous ASCs, as well as investigate the mechanisms involved.
• ADIPOA will improve the procedures significantly with respect to efficiency and safety. The added value will be provided not only by inputs from the academic participants, but also by industrial background associated with SMEs.
• SME participation will integrate the cross-sectoral academic expertise, reducing the gap between the achievement of the research results and their effective use by the economy and society.
e. Social impacts
Among the degenerative diseases associated with aging, OA is the most common pathology, affecting for example 16% of the European female population over 65 years. OA is responsible for substantial pain and functional disability. The usually
recommended treatment includes physical therapy, analgesics, local infiltration of corticosteroids and HA, without structural benefit.
• Thus, there are over 58 million Europeans that suffer from severe OA for whom no disease-modifying treatments are currently available, which constitutes a major health and societal problem (Datamonitor, Stakeholder Insight: OA - Drug development lags behind rising OA population, 2009).
• ADIPOA will definitively test, via a multicentre clinical trial, procedures that should achieve tissue repair in the long-term, to the benefit of a great number of patients with OA.
• If successful, this treatment will greatly improve the quality of life of sufferers.
• It will also address the socioeconomic costs associated with disability, long-term medical care, hospitalisation, and lost work performance.
• In addition, the development of therapeutic applications of cell therapy technology should drive new job opportunities in the EU within SMEs, research institutes, and clinical settings. Once the technology becomes fully commercialised, it should lead to facilities with highly trained staff in every major university hospital across Europe.

Osteoarthritis (OA) is the most common musculoskeletal disorder in adults leading to the progressive loss of articular cartilage and most frequently pain and disability. The lifetime prevalence for OA in Germany is estimated 22.1% (26.6 female; 17.3% male - 2009) and strongly correlates with age. Hence, numbers are estimated to duplicate within the next 20 years in respect to the demographic change in western population. Based on WHO prevalence data, there were 5,023,400 persons with OA of the knee joint in the EU in 2000. OA affects over 70 million EU citizens and is responsible for over 80 billion €/a in cost and lost productivity. These data underline the socio-economic impact of OA treatment reflecting an approx. 3.2% (8 billion €) of all direct health cost in Germany. The data basis for OA cell based therapies is poor and therefore, controlled clinical trial showing safety and efficacy are of major importance. Our first interim results of the ADIPOA trial are promising and make it urgently necessary to validate the efficacy within a larger OA cohort. Funding for the ADIPOA 2 phase II/III clinical trial has been applied within the EU Horizon 2020 Health Grant. A successful conformation of the ASC based therapy concept could be of an important step to establish an alternative therapy option for OA patients in Europe.

f. Main dissemination activities
International per reviewed original papers:
The Adipoa project leads to the publication of many international per reviewed papers.


For coming publications:


Oral communications at international meeting:
Over the 4 years of the project execution the scientific leaders involved in the consortium presented the Adipoa concept and the main results on a regular basis at European and international level.

- IOR (G. Lisignoli) : Adipose stromal cells down-modulate inflammatory factors in synoviocytes and chondrocytes from osteoarthritis patients. September 15-18, 2011 / World Congress on Osteoarthritis, San Diego USA.
- CNRS (Casteilla L.): Adipose derived stroma cells: their identity and uses in clinical trials,. May 23-25, 2012. ADIPOA symposium. Würzburg, Germany
- IOR (Lisignoli G.): Inflammatory factors released by osteoarthritic chondrocytes and synoviocytes are down-modulated by adipose stromal cells. May 12-15, 2012. 10th World Congress of the international cartilage. Repair Society. Montreal, Canada
- WU (Nöth U.) : Intraarticular injection of mesenchymal stem cells for osteoarthritis therapy. September 17, 2013. 11th World Congress of the International Cartilage Repair Society ICRS. Izmir, Turkey.
Major disseminations were realized on the scientific- as well as on public level. The ADIPOA clinical trial was presented on top-class scientific meetings (e.g. WITE 2012, World Congress of Regenerative Medicine 2012, TERMIS 2013, EFORT 2014). Symposia were organized by the ADIPOA consortium to report about progress of cell based OA therapy. Combined meetings including other EU funded projects were organized or visited to disseminate the ADIPOA project.

General public main dissemination :
On the public level, national press releases, articles in newspaper, and magazines were initiated for public dissemination. A press conference have been organised by the coordinator in Montpellier (France) in October 2012. Many important national media have been invited.

This event led to the publication of 26 articles within the following 3 months : 12 web publications, 8 press articles, 3 interviews for radio and 3 for TV.

Patent :
A patent was filed by the partner CNRS in 2012.

Modification des effets immuno-modulateurs des cellules. Y Jeanson, Roxane Blache, K Tarte, L. Casteilla (FR1255957)

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