Conclusions: The prolonged intra-articular inhibition of IL-1 reduced the severity of arthritic changes in both cartilage and joint tissue. However, the inhibition of TNF-α resulted in detrimental bone morphological changes, loss of cartilage, and inflammation of joint tissue. This study shows a novel reduction in post-trauma inflammation and demonstrates utility for an injectable drug depot for clinical intra-articular applications in the treatment of joint trauma.

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IDENTIFICATION OF THE PATIENTS WHO RESPOND SAFELY AND OPTIMALLY TO INTERVENTION WITH BIOLOGICS; LESSON LEARNED FROM RHEUMATOID ARTHRITIS

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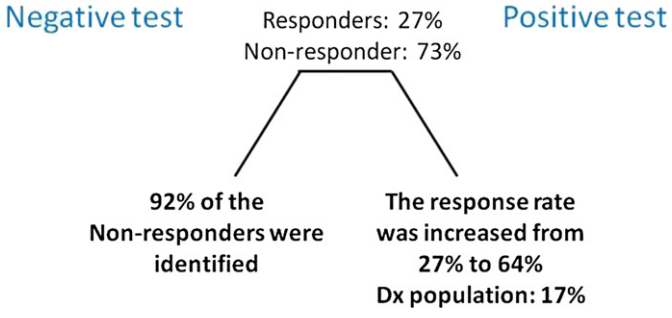
Purpose: Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized debilitating joint destruction, if not treated aggressively in the patient in most need of treatment. Personalized health care is needed and tested in RA, as response rates are low compared to the potential side effects and cost of treatment. Thus lessons on patient segregation can be learned and used in other joint diseases, such as osteoarthritis (OA). We investigated whether tissue-specific, serum-based biomarkers measured at baseline or after 1 dose could identify "super responders" to 4 mg/kg tocilizumab in patients with moderate to severe RA on a methotrexate background enrolled in the LITHE study.
Methods: The LITHE biomarker study (n=740) was a phase III study of 4 and 8mg/kg TCZ in combination with MTX. Patients were separated into ACR50 (week 52) responders and non-responder and serum biomarkers were measured at baseline and week 4. Following tissue-specific biomarkers were measured; C3M (synovial turnover), CRPM (connective tissue inflammation), C2M (cartilage degradation), CTx (bone resorption), osteocalcin (bone formation), CRP (acute phase reactant) and MMP3 (proteolytical activity). ROC was use to find the optimal cut-offs for the biomarkers at baseline and change from baseline to week4. CART analysis was used to segregate patients and 2x2 contingency test was used for identifying response rates.
Results: A simple combination of 4 baseline markers (C1M, C3M, MMP-3 and CRPM) increased the response rates (ACR50 at week 52) from 27 to 54%, while restricting the patient population to 22 %. When including the change from baseline to 4 weeks of cartilage degradation or bone formation, patient benefit was enriched to 64 %, while allowing continued treatment of 17% of patients and referral of 92% of the non-responders earlier to other possible interventions (figure).
Conclusions: By using a combination of simple serological markers, response rates were more than doubled in so-called "IL-6 super responders". This may assist in identification of the patients, in any inflammatory disease, who respond most optimally to given interventions, with fewer AEs, and thus provide a stronger risk/benefit/cost value proposition to patients and payers.



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IDENTIFICATION OF FIBROBLAST GROWTH FACTOR-18 AS A MOLECULE TO PROTECT AND REGENERATE ARTICULAR CARTILAGE

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Purpose: Aiming at the disease-modifying treatment of osteoarthritis (OA), we sought to identify genes that maintain the homeostasis of adult articular cartilage and regenerate its lesions by gene expression profile analyses.
Methods: We initially performed two sets of microarray analyses. First, to identify genes involved in maintenance of articular cartilage, we compared gene expression profiles between adult articular (AA) and adult growth plate (AG) cartilages in 10-week-old rats. Second, to identify genes involved in regeneration of articular cartilage, we compared the profiles between infant superficial (IS) and infant deep (ID) layers of epiphyseal cartilage in 6-day-old rats. For genes which were up-regulated ≥10-fold both in AA than AG and in IS than ID, we performed real-time RT-PCR for the confirmation. In vivo expression of the identified gene was examined by immunohistochemistry of articular and growth plate cartilage of 14-week-old rats. The therapeutic effect was examined in the experimental OA model by surgical induction of instability in the knee joints of adult rats. To learn the underlying mechanism, the protective ability of articular cartilage was assessed by measuring the amount of sulfated glycosaminoglycan (sGAG) released into the medium in the ex vivo culture of bilateral femoral heads of 3-week-old mice. Proliferation and migration were analyzed in the cultures of mouse articular chondrocytes using Cell Counting Kit-8 and Oris Cell Migration assay systems, respectively. Expression levels of catabolism-related factors (Mmp9, Mmp13, Adamts4, Adamts5, Timp1, Timp2, and Timp3) and anabolism-related factors (Col2a1 and aggrecan) in the cultures of mouse femoral heads and mouse articular chondrocytes were analyzed by real-time RT-PCR.
Results: Microarray analyses revealed that 40 and 186 genes had ≥10-fold higher expression ratios of AA/AG and IS/ID, respectively, and 16 genes showed ≥10-fold of both AA/AG and IS/ID ratios. The ratios of the 16 genes were confirmed to be ≥10 fold by real-time RT-PCR analysis. Among them three genes were expressed more strongly in AA than in IS. In these three genes, fibroblast growth factor-18 (Fgf18) was the extracellular and secreted factor of which the AA/AG ratio was the highest in the microarray analysis. Immunohistochemistry showed that Fgf18 was strongly expressed in the articular cartilage chondrocytes of adult rats but was hardly detected in the growth plate cartilage. In the rat surgical OA model, a once-weekly intra-articular injection of recombinant human (rh) FGF18 given 3 weeks post-surgery prevented cartilage degeneration in a dose-dependent manner at 6 and 9 weeks after surgery, with a significant effect at 10 μg/week of rhFGF18. As an underlying mechanism, rhFGF18 suppressed the sGAG release into the culture medium in the ex vivo culture of mouse femoral heads. Furthermore, rhFGF18 accelerated proliferation and migration of cultured mouse articular chondrocytes. Among catabolic and anabolic factors, rhFGF18 decreased Adamts4 and increased Timp1 expressions in the cultures of mouse femoral heads and murine articular chondrocytes, while it decreased Col2a1 and aggrecan expressions in both cultures.
Conclusions: The present gene expression profiling analysis identified Fgf18 as a molecule to protect and regenerate adult articular cartilage, causing prevention of OA development by the intra-articular injection in a rat model. This effect may be mediated by inhibition of cartilage catabolism, and acceleration of proliferation and migration of articular chondrocytes, indicating a possible disease-modifying OA treatment.

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MICRORNAS ARE PROGNOSTIC MARKERS FOR THE CHONDROGENIC POTENTIAL OF MSCS

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Purpose: The capacity of mesenchymal stromal cells (MSCs) to differentiate into chondrocytes as well as to function as trophic mediators restoring joint homeostasis make them a promising cell source for a disease modifying treatment in osteoarthritis. MSCs can be easily harvested from various locations of the body, including amongst others bone marrow, periosteum, synovium, synovial fluid, adipose tissue, buccal fat pad, infrapatellar fat pad and osteoarthritic cartilage. MSCs are a heterogeneous cell population and large inter-donor variation with respect of the chondrogenic potential of these cells has been reported which may hamper clinical application. Presently, prognostic

markers predicting the chondrogenic differentiation potential of culture expanded MSCs derived from multiple donors are lacking. The objective of this study is to identify such prognostic markers.
Methods: In this study 20 human MSC donors were tested for their ability to produce cartilage in a standard chondrogenic differentiation assay consisting of pellet culture in the presence of serum free medium and TGFβ. Cartilage formation was scored on the basis of histological matrix formation, mRNA expression levels of chondrogenic marker genes and quantification of glycosaminoglycan deposition. Of each of these donors genome wide mRNA expression profiles were obtained using an affymetrix microarray platform before the onset of differentiation. In addition, small nucleotide RNAs were isolated for miRNA profiling using a panel of miRNAs previously implemented in chondrogenesis.
Results: Only 3 donors out of 20 were identified as donors with high chondrogenic potential, whereas 9 showed moderate and 8 low chondrogenic potential. Despite these huge differences in chondrogenic potential, genome wide mRNA profiling at the onset of differentiation showed only marginal differences between the 3 groups. In contrast, profiling of microRNAs (miRNAs) previously implemented in chondrogenesis and cartilage homeostasis showed a very distinctive pattern between good and bad performing donors. We also studied the changes in miRNA expression during a 7 day differentiation period of MSCs in pellet culture and identified *miR-210* and *miR-630* as positive regulators of chondrogenesis with *miR-630* as a potential marker for high performing donors. In contrast *miR-181* and *miR-34a*, both of which are negative regulators of chondrogenesis, were up-regulated during differentiation in bad performing donors.
Conclusions: In contrast to the marginal differences at the global mRNA level between good and bad MSC donors with respect of chondrogenic differentiation potential, screening of a panel of miRNAs previously implemented in cartilage formation showed more clear segregation between good and bad performing donors. MiRNA profiling of MSC donors may, therefore, have prognostic value to select MSC donors with respect of their chondrogenic differentiation potential and their capacity to restore cartilage homeostasis after intra-articular injection in the osteoarthritic joint.

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THE REPAIR OF FOCAL CARTILAGE DEFECTS USING HUMAN EMBRYONIC STEM CELLS

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Purpose: Damage to articular cartilage occurs frequently as a result of joint trauma and disease. Cell based treatments to repair defects have been developed using autologous chondrocytes and bone marrow stem cells and have shown some success. We have investigated the potential of human embryonic stem cells (hESC) as a source of chondrocytes, as they have capacity for unlimited self-renewal and could provide a ready supply of donor cells. In initial work we developed a 14 day culture protocol using serum free, chemically defined medium and generated chondro-progenitors from hESC, which were up to 97% SOX9 positive, expressing COL2A1 and ACAN genes. This system is chemically defined and scalable and with potential to provide cells for clinical grade use. In this study we developed the protocol further and tested hESC derived chondro-progenitors in vivo in focal defects in immunocompromised nude rats.
Methods: Human embryonic stem cells (hESC) expanded on feeder culture were transferred to feeder free/ serum free culture on fibronectin coated plates. After two passages a 14 day chondrogenesis protocol was initiated with a sequential series of growth factors, which drive the hESCs through mesendoderm/mesoderm to chondroprogenitors. These cells were characterised by qRT-PCR using a range of chondrocyte specific marker genes, negative controls and by immunofluorescence for SOX9, a chondrocyte transcription factor. Some chondro-progenitors were also derived from hESC transduced with GFP using lentiviral vectors. To test their capacity for cartilage formation, chondro-progenitors in fibrin gel (3X106 cells/ml) were implanted into osteochondral defects (2mm diameter, 2mm deep, 14 defects in 8 animals) in the patella groove of nude rats. Joint tissue was removed, decalcified, fixed and sectioned for histological and immunochemical analysis after 4 weeks or 12 weeks.
Results: The hESC derived chondro-progenitors at the end of the protocol formed cell clusters and showed chondrocyte properties, including high expression of SOX9. They strongly immunostained for SOX9 protein and for collagen II and for aggrecan and expressed collagen II and XI genes, but not collagen I and negligible collagen X, which is a marker for hypertrophic chondrocytes. The expression of matrilin 3 increased and was more than 50 time higher than matrilin 1 at the end of the protocol, again suggesting an articular rather than an epiphyseal phenotype. Also, we found the expression of core band factor beta (CBF-beta) was elevated and ZNF145, ZNF219, p300 and SirT1 were also increased. Defects in nude rat joints were seeded with chondro-progenitors (approx 2 x105cells per defect) in fibrin gel and in a contralateral control, fibrin gel alone. Joint tissue was isolated at 4 weeks and 12 weeks and in these preliminary experiments histology showed evidence of repair cartilage filling in the defect areas in the cell seeded joints (cartilage in 2 from 3 animals at 4 weeks and in 2 from 4 animals at 12 weeks). When GFP cells were implanted they were detected by fluorescence within areas of neo-cartilage formation and immunohistology using anti human vimentin antibody confirmed human cells in the repair tissue, which stained with safranin O for proteoglycan and immunostained for collagen II. Only fibrous tissue was found in the defect areas of joints implanted with fibrin gel only.
Conclusions: Human embryonic stem cells in feeder free, serum free, chemically defined medium were differentiated into chondrogenic cells, which when implanted in focal defects in nude rats participated in the formation of cartilage repair tissue assessed up to 12 weeks. The study demonstrates that human embryonic stem cells can be efficiently differentiated to produce chondro-progenitors with a protocol that is suitable for future clinical applications.

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DISEASE MODIFYING, RECEPTOR SELECTIVE ANALOGS OF FGF18 FOR GLOBAL, ARTICULAR CARTILAGE PRESERVATION AND REPAIR IN EXPERIMENTAL OSTEOARTHRITIS

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Purpose: We aimed to study the benefits and potential adverse effects of FGF18 and FGF18 analogs on joint articular cartilage preservation in an experimental model for Osteoarthritis (OA).
Methods: A series of genetically engineered isoforms of FGF18, a major cartilage growth and differentiation factor, were tested in a murine model for surgically induced Osteoarthritis. OA was induced by transsection of the anterior medial meniscotibial ligament in the knee joint, which causes destabilization. Ligands were selected for their FGF receptor specificity, discriminating FGFR2 from FGFR3 signaling in bone and cartilage. Stabilization and controlled release of the growth factors were achieved through chemical conjugation to Hyaluronic acid (HA) which were further enhanced using a novel Fibrin-HA based hydrogel. Ligands were administered by repeated intra-articular injections into the murine knee joints according to various regimens.
Results: The capacity of these factors in the preservation and tissue repair of the various articular cartilage compartments was evident by histology and gross morphology. HA link enhanced the potency of FGF18. However, wild type FGF18, particularly when administered as soluble, free ligand, significantly enhanced osteophyte formation and size at all doses tested. The soluble FGFR3 selective ligand, on the other hand, did not induce osteophytes formation. Unexpectedly, the Fibrin-HA hydrogel by itself conferred a protective effect against damage induced OA.
Conclusions: Our results demonstrate a differential effect of FGF18 analogs different in their FGF receptor activation on cartilage preservation and remodeling in a murine model of damage induced OA. More specifically we suggest that FGF18 signaling via FGFR2 may be the primary trigger for enhanced local osteogenic effect and osteophyte formation.

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LOCATION-INDEPENDANT ANALYSIS OF INTRAARTICULAR SPRIFERMIN EFFECTS ON CARTILAGE STRUCTURE USING ORDERED VALUES

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