a therapeutic target in RA and OA. To date, there are no published data on the functionally distinct VEGF-A splice variants in either RA or OA.

Objectives: To determine the patterns of, and relationships between, VEGF-A, SRPK1, and SRSF1 expression and activation and synovial inflammation in human RA and OA.

Methods: The study was approved by the Nottingham Research Ethics Committee 1 (05/Q2403/24) and Derby Research Ethics Committee 1 (11/H0405/2). Tissues were selected from age- and sex-matched cases in the University of Nottingham joint tissue repository. Post-mortem (PM) samples of healthy knee synovium (n=14, no history of arthritis or knee pain in the 12 months prior to death, no significant articular or synovial pathology) and arthroplasty-derived synovium samples from OA (n=35) or RA (n=14) patients were compared. OA samples were selected to represent the variety of inflammation levels, from low to high grade (0-3, Haywood et al., 2003). 8µm thick sections were stained for SRSF1, SRPK1, total VEGF-A and VEGF-A_{xxx}b via immunohistochemistry. Expression was estimated as fractional area, relative staining intensity (VEGF-A_{xxx}b), and SRSF1 activation quantified by the degree of nuclear localisation. Statistical analyses were performed using Kruskal-Wallis followed by Dunn's tests and Spearman's rank correlations.

Results: SRPK1 expression was similar across all conditions. SRSF1 showed significantly higher expression in the OA tissue compared to PM (H(2)= 11.29, p=0.002; OA median=0.2, IQR(0.15, 0.28); PM median=0.09, IQR(0.07, 0.16)), and significantly higher nuclear localisation (indicating activation) in RA vs. OA, and in both RA and OA vs PM (H(2)=37.65, p<0.0001 RA cf. PM; p=0.007 OA cf. PM; RA median=89, IQR(83, 93); OA median=36.1, IQR(29, 42); PM median=19.8, IQR(14,21)). Nuclear SRSF1 was significantly correlated with inflammation score (r= 0.52, p<0.05). Total VEGF-A expression was significantly increased in RA compared to PM and OA (H(2)=23.3, p<0.001 RA cf. PM; RA median=0.4, IQR(0.37,0.59); PM median=0.18, IQR(0.15,0.2)) and was also correlated with the severity of inflammation (r=0.47 p<0.05). VEGF-A_{xxx}b showed no change in expression in OA or RA, although VEGF-A_{xxx}b staining intensity was significantly higher in RA samples, compared to controls (H(2)=7.2 p=0.02; RA median=2.3(1, 4); PM median=0.9 (0.7, 1.4)).

Conclusion: Increased levels of SRSF1 activation, and the association of total VEGF-A expression with inflammation score, support the hypothesis that there is activation of alternative splicing in inflamed synovium in RA and OA. Targeting this pathway could be a novel therapeutic strategy in OA & RA.

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Both companies have with a focus on development of alternative RNA splicing control for therapeutic application in a number of different conditions, including ophthalmology (www.exonate.com), analgesia and arthritis (www.emendatherapeutics.com)., Consultant of:

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AB0067

CHONDROCALCINOSIS IS ASSOCIATED WITH A SPECIFIC EFFECT ON THE CHONDROCYTE PHENOTYPE THAT MARKEDLY DIFFERS FROM OA

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Background: Calcification of cartilage with BCP crystals is a common finding during osteoarthritis (OA) and is directly linked to the severity of the disease and hypertrophic differentiation of chondrocytes. Chondrocalcinosis (CC) is associated with CPPD crystal formation. There is only little knowledge about the effect of CPPD crystals on chondrocytes.

Objectives: The aim of this study was to investigate the chondrocyte phenotype in CC cartilage and the effect of CPPD crystals on chondrocytes.

Methods: Cartilage samples of patients with CC were used and compared with samples of severe OA patients without chondrocalcinosis and healthy cartilage samples served as control. Radiological presence of chondrocalcinosis was evaluated using standard X-ray pictures, as well as macroscopically inspection. The cartilage samples were stained using von Kossa/Safranin-orange staining. These stainings were used for OA severity scoring using the Chambers-Score. FTIR analyses were performed to distinguish CPPD and BCP crystals in cartilage. Chondrocyte differentiation markers were evaluated using Collagen 2 and X, as well as Sox9 and aggrecan as markers for chondrocyte hypertrophic differentiation in immunohistochemistry and qRT-PCR. TUNEL staining was performed to investigate cell death. In vivo results were validated using qRT-PCR for the expression of the respective genes after stimulation of C28 chondrocytes with CPPD and BCP crystals.

Results: Radiologically detectable cartilage calcifications were evident in chondrocalcinosis patients, but absent in OA patients without CC. CPPD crystals were detected on the cartilage surface, whereas BCP crystals were detected in the pericellular matrix of hypertrophic chondrocytes. CC cartilage exhibited an increased collagen X expression compared to healthy cartilage, as well as to severe OA cartilage containing BCP calcification. Interestingly, aggrecan and collagen 2 were not reduced in CC cartilage, but markedly reduced in OA cartilage. TUNEL positive cells were significantly increased in CPPD cartilage compared to OA cartilage, although the histological OA severity was lower. qRT-PCR indicated no relevant influence of CPPD crystals on hypertrophic marker genes, whereas BCP crystals significantly induced hypertrophic differentiation.

Conclusion: BCP and CPPD crystals seem to trigger differential effects on the chondrocyte phenotype. BCP crystals induce hypertrophic differentiation, which is not induced by CPPD crystals.

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AB0068

NOVEL CHONDROPROTECTIVE AND ANTI-INFLAMMATORY EFFECTS OF THE SELECTIVE HUMAN MELANOCORTIN MC3 RECEPTOR AGONIST PG-990 ON SNAP ACTIVATED CHONDROCYTES

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Background: Osteoarthritis (OA) is a degenerative joint disease that affects over 250 million people worldwide [1] with treatments focussing on the symptoms rather than the cause of the pathology [2, 3]. Thus, this degenerative joint disease requires novel treatment options [3, 4].

Therefore, the melanocortin system [4] could provide a novel avenue to explore given its ability to exert anti-inflammatory effects and chondroprotection [5], although the receptor subtype involved is unclear.

Objectives: This study aims to assess the chondroprotective and anti-inflammatory effects of the selective human melanocortin MC1 receptor agonist BMS-470539 dihydrochloride and the selective human MC3 receptor agonist PG-990 on S-Nitroso-N-acetyl-DL-penicillamine (SNAP) activated chondrocytes.

Methods: The human chondrocytic cell-line C-20/A4 was seeded at 25.0×10^6 viable cells/ml (5 μ l droplet was transferred into individual wells of a 96-well plate). Micromass cultures [6] were stimulated with SNAP (1.0 mM) and after 2h treated with Dexamethasone (1.0 μ M), selective human melanocortin MC1 receptor agonist BMS-470539 dihydrochloride (10.0 μ g/ml) or selective human melanocortin MC3 receptor agonist PG-990 (10.0 μ g/ml) for 6h. Cell viability was determined by MTT assay, Caspase -3 and -7 activity determined by Caspase-Glo 3/7 apoptosis assay. Glycosaminoglycan (GAG) content determined by alcian blue staining and anti-inflammatory heme-oxygenase-1 (HO-1) protein expression was determined by western blot. Data are expressed as Mean $\pm$ S.E.M of $n=4$ samples repeated in triplicate. $\#p \leq 0.05$ vs control or $*p \leq 0.05$ vs stimulus.

Results: Cell viability analysis showed SNAP stimulation caused a maximal cell death of 23% ($\#p \leq 0.05$). Dexamethasone, BMS-470539 dihydrochloride and PG-990 inhibited cell death by 2%, 98% and 129% respectively ($*p \leq 0.05$). SNAP stimulation caused a significant increase in Caspase -3 and -7 activity, which was inhibited by Dexamethasone, BMS-470539 dihydrochloride and PG-990 by 8%, 5% and 19% respectively ($*p \leq 0.05$). GAG content was significantly reduced by SNAP by 29% ($\#p \leq 0.05$), which was inhibited by Dexamethasone, BMS-470539 dihydrochloride and PG-990 by 1%, 3% and 14% respectively ($*p \leq 0.05$). SNAP also caused a significant decrease in HO-1 protein expression, which was increased by Dexamethasone, BMS-470539 dihydrochloride and PG-990 by a 1.0-fold, 1.1-fold and 2.1-fold increase respectively ($*p \leq 0.05$).

Conclusion: The selective human melanocortin MC3 receptor agonist PG-990 exhibited enhanced chondroprotection and modulation of inflammatory and tissue destructive mediators following SNAP activation compared to Dexamethasone and the selective human melanocortin MC1 receptor agonist BMS-470539 dihydrochloride. This suggests that melanocortin peptides display enhanced chondroprotective and anti-inflammatory effects at the MC3 receptor sub-type in this cell line.

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AB0069

LORECIVIVINT (SM04690), AN INTRA-ARTICULAR, SMALL-MOLECULE CLK/DYRK1A INHIBITOR THAT MODULATES THE WNT PATHWAY, AS A POTENTIAL TREATMENT FOR MENISCAL INJURIES

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Background: Meniscal injuries, associated with pain, stiffness, and localized swelling, are the most common pathology of the knee with a prevalence of 61 per 100,000.¹ Meniscal damage is a frequent finding on MRI images of knee osteoarthritis (OA)²; while a meniscal tear can lead to knee OA, knee OA can also lead to a spontaneous meniscal tear.³ Efforts to repair meniscal damage have been largely unsuccessful and do not prevent the progression of degenerative changes that lead to knee OA.⁴ The Wnt signaling pathway has been shown to be regulated during meniscal development,^{5,6} suggesting that manipulation of this pathway may influence the regenerative capacity of the meniscus. Lorecivivint (LOR; SM04690) is an intra-articular (IA), small-molecule CLK/DYRK1A inhibitor that modulates the Wnt pathway.

Objectives: LOR was evaluated in preclinical studies to determine its protective and anabolic effects in ex vivo explants and in a rat model of chemically induced inflammatory meniscus degeneration.

Methods: Effects of LOR (30 nM) on expression of matrix metalloproteinases (MMPs) in cultured rat menisci treated with IL-1B were measured by qPCR. In vivo, LOR activity was evaluated in a rat model of monosodium iodoacetate (MIA) injection-induced inflammatory meniscus degeneration. A single IA injection of MIA was immediately followed by a single IA injection of LOR (0.3 μ g) or vehicle. Knees were harvested on Days 1, 4, and 11 and menisci were isolated. Anti-inflammatory effects were evaluated by measuring *TNFA* and *IL6* expression by qPCR. Meniscus protection was evaluated by qPCR for MMPs and aggrecanase and anabolic effects by qPCR for collagens.

Results: In ex vivo meniscal explants, LOR inhibited expression of *MMP1*, *MMP3*, and *MMP13* compared to DMSO ($P < 0.01$). In vivo, LOR significantly decreased expression of these MMPs and aggrecanase ($P < 0.05$) compared to vehicle in the rat model of inflammatory meniscus degeneration at Day 4 after MIA injection. In addition, LOR reduced expression of inflammatory cytokines *TNFA* and *IL6* at Day 4 compared to vehicle. Finally, LOR increased expression of collagen types I, II, and III at Day 11 after MIA injection.

Conclusion: LOR exhibited protective effects in the meniscus ex vivo and in vivo by reducing the expression of catabolic enzymes compared to control. Anti-inflammatory effects of LOR were demonstrated by inhibition of inflammatory cytokine expression. Compared to vehicle, LOR increased expression of collagens in vivo, indicating potential meniscal anabolic effects. These data support further investigation of LOR as a potential disease-modifying therapy for meniscal injuries.

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AB0070

LORECIVIVINT (SM04690), A POTENTIAL DISEASE-MODIFYING TREATMENT FOR KNEE OSTEOARTHRITIS, DEMONSTRATED CARTILAGE-PROTECTIVE EFFECTS ON HUMAN OSTEOARTHRITIC EXPLANTS

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Background: Wnt pathway upregulation contributes to knee osteoarthritis (OA) through osteocyte differentiation, cartilage thinning, and inflammation. Lorecivivint (LOR; SM04690), a novel, small-molecule CLK/DYRK1A inhibitor that modulates the Wnt pathway, demonstrated disease-modifying potential for knee OA in preclinical studies.¹ However, the specific mechanisms by which LOR protects cartilage in knee OA are unclear.

Objectives: To evaluate the cartilage-protective effects of LOR on human OA explants from total knee replacement (TKR) donors.

Methods: Knee joint tissue from 22 TKR donors was obtained. IRB approval was obtained from Scripps Health. Cartilage was scored using the Outerbridge classification system based on gross appearance (grade 1=least-damaged tissue, grade 4=most-damaged tissue). Cartilage explants (4 mm in diameter) with Outerbridge grades 2–3 were harvested and cultured for 48 hours to reach metabolic stability. They were then treated with LOR (10 nM, 30 nM) or DMSO and stimulated with either IL-1 β (10 ng/ml) or TNF- α (20 ng/ml)+oncostatin M (OM) (10 ng/ml) or left unstimulated. After 72 hours, supernatants and explants were collected. Gene expression of matrix metalloproteinases (MMPs) 1, 3, and 13 was measured by qPCR and protein levels of MMP-1, MMP-3, MMP-13, and thrombospondin-motif-containing disintegrins/metalloproteinases ADAMTS-4 and ADAMTS-5 were measured in supernatants by ELISA. Glycosaminoglycan (GAG) and nitric oxide (NO) levels were measured in supernatants using the dimethylmethylene blue assay and Griess assay, respectively. One-way ANOVA was used for multiple group comparisons.

Results: Treatment with IL-1 β or TNF- α +OM led to statistically significant increases in gene expression of *MMP1*, *MMP3*, and *MMP13* and increased secretion of GAG, MMP-1, MMP-3, MMP-13, ADAMTS-4, ADAMTS-5, and NO in supernatants compared to unstimulated control. Treatment with LOR decreased both IL-1 β -stimulated and TNF- α +OM-stimulated gene expression of *MMP1*, *MMP3*, and *MMP13* and secretion of GAG, MMP-1, MMP-3, MMP-13, ADAMTS-4, ADAMTS-5, and NO in supernatants compared to treatment with DMSO.

Conclusion: LOR demonstrated potent inhibition of cartilage catabolism enzyme production in human OA explants compared to controls. These cartilage-protective effects support the development of LOR as a potential disease-modifying treatment for knee OA. Human trials are ongoing.

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