were stimulated on the same day of isolation. Both moDCs and mDCs were pre-treated with Tofacitinib and then stimulated with either lipopolysaccharide (LPS) or combination of LPS with IFN-γ for 4 hours. Cytokines were measured using enzyme-linked immunosorbent assay (ELISA) and gene expression was assessed using quantitative polymerase chain reaction (qPCR).
Results: Treatment of both mDCs and moDCs with Tofacitinib led to a decreased mRNA expression of IL-12 p40 (*IL12B*) in the presence of TLR4 and IFNγ co-stimulation. The decreased *IL12B* mRNA expression also resulted in lower production of IL-12 p40 and IL-23 proteins in mDCs.
Conclusion: In this work, we demonstrated for the first time that Tofacitinib can suppress the production of IL-23/IL-12 p40 subunit in mDCs, upon the condition that an active type II IFN signalling is also present in these cells. This observation indicates that specific factors, such as endogenous IFN-γ levels in the serum of PsA patients, can possibly predict differential responses to Tofacitinib treatment.
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OA, aetiology, pathology and animal models

AB0063 **AGING CARTILAGE IN WILD-TYPE MICE: AN OBSERVATIONAL STUDY**

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Background: Many animal models of osteoarthritis (OA) have been used to study the pathogenesis of cartilage degeneration[1]. In mice, spontaneous OA can occur in wild-type or genetically modified animals. The first report of spontaneous OA developing in wild-type mice was published in 1956 [2] and changes affecting the knee joint were further related to OA by using ultrastructural- histochemical analyses. However, a quantitative assessment of age-related evolution of OA-type cartilage lesions is lacking. The OA Research Society International (OARSI) grading score was adapted to semi-quantify histopathogical changes occurring in OA animal models, including mice [3]. The OARSI score has been used to describe changes occurring in induced or genetic OA mouse models but not to describe spontaneous age-related evolution of OA-type cartilage lesions in wild-type mice.
Objectives: We aimed **t**o describe the spontaneous evolution of age-related changes affecting knee joint articular cartilage, walking speed and a serum biomarker of cartilage remodeling in C57BL/6 wild-type male mice.
Methods: Histological changes were assessed by the OARSI score in newborn, 1-week- and 1-, 3-, 6-, 9- and 12-month-old C57BL/6 wild-type male mice, walking speed by the Locotronic system, and serum C-terminal telopeptide of type II collagen (CTX-II) content by ELISA in 1-, 3-, 6-, and 9-month-old C57BL/6 wild-type male mice.
Results: Mean (SD) OARSI score increased from 0.2(0.3) to 1.3(0.6) (p=0.03) between 1 and 3 months of age and from 1.3(0.6) to 3.3(0.6) (p=0.04) between 3 and 6 months of age. Mean walking speed was stable between 1 and 6 months of age but decreased from 11.4(1.8) to 3.2(0.8) cm.s-1 (p=0.03) between 6 and 9 months of age. Serum CTX-II content was maximal at 1 month of age, then decreased from 12.2(8.5) to 2.4(8.4) pg/ml (p=0.02) between 1 and 3 months of age, remaining low and stable thereafter.
Conclusion: C57BL/6 wild-type male mice showed continuously increasing osteoarthritic changes but delayed decreasing walking speed with age. These variations were maximal between 3 and 9 months of age. Maximal serum CTX-II content preceded these changes.
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Table 1. Evolution of cartilage changes, walking speed and serum C-terminal telopeptide of type II collagen (CTX-II) concentrations in wild-type C57BL/6 male mice.

Age	New-born	1 week	1 month	3 months	6 months	9 months	12 months
OARSI score (0 to 6)	0.0 (0.0)	0.0 (0.0)	0.2 (0.3)	1.3 (0.6)*	3.3 (0.6)*	3.7 (0.6)	4.3 (0.6)
Walking speed ($cm.s^{-1}$)	-	-	10.5 (1.5)	11.3 (4.3)	11.4 (1.8)	3.2 (0.8)*	-
CTX-II concentrations (pg/ml)	-	-	12.2 (8.5)	2.4 (8.4)*	1.1 (4.0)	4.0 (3.8)	-

N ≥ 3 per timepoint. All results are means (standard deviation). *p<0.05 as compared to the previous timepoint using the non-parametric Mann-Whitney U-test.

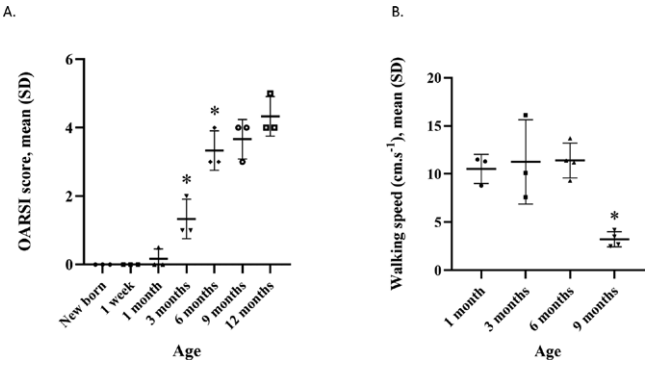

Figure 1. (A) New born,1-week- and 1-, 3-, 6-, 9- and 12-month-old wild-type C57BL/6 male mice (N=3 per timepoint) knees were evaluated for cartilage changes following OARSI recommended guidelines by 3 independents readers. Each point represents the mean of OARSI score per mice as scored by 3 independent readers. **(B)** 1-, 3-, 6- and 9-month-old wild-type C57BL/6 male mice were evaluated for walking speed using the Locotronic® system. Each point represents the mean of 3 measures of walking speed per mice. All results are means (SD). *p<0.05 as compared to the previous timepoint using the non-parametric Mann-Whitney U-test.

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AB0064 **CHANGES IN THE VASCULAR ENDOTHELIAL GROWTH FACTOR A (VEGFA) SPLICING AXIS IN HUMAN SYNOVIUM ARE RELATED TO INFLAMMATION IN ARTHRITIS**

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Background: VEGF-A is a key regulator of rheumatoid (RA) and osteoarthritis (OA). During articular inflammation in OA and RA there is increased synovial angiogenesis and upregulation of angiogenic growth factors such as VEGF-A. VEGF-A comprises two splice variant families, VEGF-A_{xxx}a and VEGF-A_{xxx}b (xxx represents the number of amino acids, from 121 to 206), resulting from alternative splice site selection in exon 8. This site selection is controlled by Serine/Arginine Rich Splicing Factor Kinase 1 (SRPK1), which phophorylates Serine/Arginine Rich Splicing Factor 1 (SRSF1), inducing it to translocate to the nucleus. In most normal tissues, VEGF-A_{xxx}b isoforms predominate, with anti-nociceptive and anti-angiogenic functions. I contrast, in pathological conditions such as inflammation and solid tumours, VEGF-A_{xxx}a isoforms predominate, with pro-nociceptive and pro-angiogenic functions. VEGF-A has been proposed as

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). 8um thick sections were stained for SRSF1, SRPK1, total VEGF-A and $VEGF_{xxx}b$ via immunohistochemistry. Expression was estimated as fractional area, relative staining intensity ($VEGF\text{-}A_{xxx}b$), 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 ($r=0.47$ $p<0.05$). $VEGF\text{-}A_{xxx}b$ showed no changed in expression in OA or RA, although $VEGF\text{-}A_{xxx}b$ staining intensity was significant 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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2019: Consultancy to Pfizer (payment to University).
2018: Consultancy to Pfizer. 07.12.18. USA. 1 day. Tanezumab (payment to University).
2018: Consultancy to Pfizer. 23.11.18. Manchester UK. 1 day. Tanezumab (payment to University).
2018: Consultancy to Pfizer. 1.11.18. Skype. 4h. Tanezumab (payment to University).
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2017: Consultancy to Pfizer Ltd; personal financial disclosure
2017: Consultancy to Pfizer Ltd through Nottingham University; non-personal financial disclosure (payment to University).
2015: Consultancy to GSK Consumer Healthcare; personal financial disclosure., Speakers bureau: 2019: Irish Society of Rheumatology: speaker fees (personal pecuniary), Lucy Donaldson Shareholder of: LFD is a co-inventor on patents protecting alternative RNA splicing control and VEGF-A splice variants for therapeutic application in a number of different conditions. LFD is also a founder equity holder in, and consultant to, Exonate Ltd, and Emenda Therapeutics Ltd. 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 was 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.