Conclusion: To our knowledge, this is the first report of SM04690 inhibition of nuclear kinases CLK2 and DYRK1A, leading to effects on the Wnt pathway, chondrocytes, and inflammation (**Figure 1**). This dual mechanism of SM04690 potentially modifies OA through increased chondrocyte differentiation and function and benefits symptoms through anti-inflammatory activity. Human trials are ongoing.

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OP0073 ESTABLISHMENT OF HUMAN INDUCED PLURIPOTENT STEM CELL-LINES (IPSC) FOR IN VITRO MODELLING HAND OSTHEOARTHRITIS

Rocío Castro-Viñuelas^{1,2}, Clara Sanjurjo-Rodríguez^{1,3}, María Piñeiro-Ramil^{1,2}, Tamara Hermida Gómez², Isaac Fuentes-Boquete^{1,2}, Javier de Toro-Santos^{1,2}, Francisco J. Blanco², Silvia María Díaz-Prado^{1,2}. ¹University of A Coruña, Physiotherapy, Medicine and Biomedical Sciences, A Coruña, Spain; ²Institute of Biomedical Research of La Coruña, A Coruña, Spain; ³Leeds Institute of Rheumatic and Musculoskeletal Disease, University of Leeds, Leeds, United Kingdom

Background: Research results in the field of hand OA are currently limited due to the unavailability of tissue samples and lack of animal models replicating the features of the disease in humans. Cellular *in vitro* models are important tools to elucidate molecular mechanisms and pathways that are involved in hand OA. Specifically, induced pluripotent stem cells (iPSc) are considered ideal tools for this purpose since they allow the use of unlimited cells with chondrogenic differentiation potential. However, there are not studies published generating iPSc from patients with hand OA.

Objectives: To generate and characterize iPSc-lines from patients with radiographic hand OA and healthy donors, which can be used as cellular models of the disease, for studying the pathogenesis of the disease *in vitro* and for testing new drugs.

Methods: Patients with hand OA (non erosive hand OA with right thumb OA) and a healthy control were selected for the study. Using the explant culture technique, fibroblasts from skin biopsies of these patients were isolated. Transcriptional factors Oct4, Sox2, Klf4 and c-Myc were used for the reprogramming process; which was performed by using a non-integrating method, the Sendai virus. Cell lines obtained were morphologically, phenotypically and functionally characterized. To evaluate whether these iPSc lines could be used as cellular model of hand OA, presence of single nucleotide polymorphisms (SNPs) within the genes ALDH1A2 and SMAD3 were studied by Sanger sequencing, before and after reprogramming. Variants rs3204689 and rs12901499 respectively have been associated with severe OA of the hand (Styrkarsdottir *et al.*, 2014; Shu-Thao Gao *et al.*, 2018). Finally, chondrogenic differentiation capacity of the "healthy" and "ill" iPSc-lines was studied by means of histological techniques.

Figure 1. (A) Bright field images and images after alkaline phosphatase staining (AP staining) of iPSc colonies from each cell line. x4 magnification (B) Immunofluorescence images of pluripotency markers (NANOG and TRA-1-81) of representative clones from each cell line. Scale 100 µm. (C) Immunofluorescence images of endoderm, mesoderm and ectoderm markers in each one of the cell lines. Scale 100 µm. (D) Summary of the results obtained after single nucleotide polymorphism analysis. (E) Hematoxylin-Eosin (HE), Masson's Tricrome (TM) and Safranin-O (SO) staining after chondrogenic differentiation of the iPSc lines. x10 magnification.

Results: Fibroblasts were isolated from one patient with radiographic hand OA and one healthy donor. Three weeks after reprogramming, embryonic stem cell-like colonies emerged in culture. These cells showed positivity for alkaline phosphatase activity (fig. 1A) and the pluripotency markers Tra1-81 and Nanog (fig. 1B). Molecular analyses showed high relative expression levels of the pluripotency-related genes OCT4, SOX2, NANOG and CRIPTO in the iPSc. These cells were also able to give rise to cells from the three germ layers (fig. 1C). Indeed, during mesodermal differentiation, spontaneously beating cardiomyocytes were seen in culture. Regarding SNPs studies, cells from the patient with hand OA were homozygous for the at-risk allele in both genes studied, both before and after reprogramming (fig. 1D). The "ill" iPSc-line (MOAFIPS 15/645#7) showed worse chondrogenic differentiation than the "healthy" iPSc-line (NFIPS 15/637#7), as shown by the micromasses collagen and proteoglycan content (fig. 1E).

Conclusion: The generation of one iPSc-line from patients with hand OA is reported for the first time. The presence of the at-risk alleles within the ALDH1A2 and SMAD3 genes were maintained after fibroblast reprogramming. The iPSc lines obtained shown differences in their chondrogenic differentiation capacity, showing their usefulness to model hand OA *in vitro*, and to deeper study the role of these genetic variants in the pathogenesis of hand OA.

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OP0074 TOFACITINIB PROMOTES FUNDAMENTAL PROCESSES OF BONE HEALING

Timo Gaber^{1,2}, Antonia Brinkman¹, Alexandra Damerau^{1,2}, Moritz Pfeiffenberger^{1,2}, Lisa Ehlers^{1,2}, Frank Buttgerit^{1,2}, Paula Hoff^{1,3}. ¹Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Department of Rheumatology and Clinical Immunology, Berlin, Germany; ²German Rheumatism Research Centre (DRFZ) Berlin, a Leibniz Institute, Berlin, Germany; ³Endokrinologikum Berlin, Berlin, Germany

Background: Inflammatory diseases like rheumatoid arthritis (RA) and anti-inflammatory treatment of RA with glucocorticoids (GC) or NSAIDs negatively influence bone metabolism and fracture healing. Janus kinase (Jak) inhibition with tofacitinib has been demonstrated as a potent anti-inflammatory therapeutic agent in the treatment of RA but its impact on the fundamental processes of bone regeneration such as recruitment of human mesenchymal stromal cells (hMSCs) and chondrogenesis, osteogenesis and osteoclastogenesis is still controversial and in part elusive.

Objectives: Therefore, in this study, we aim to examine the effects of Tofacitinib on processes of bone healing under reduced oxygen availability mimicking the *in vivo* situation of the fracture gap.

Methods: To this end, we analyzed the influence of Tofacitinib on the (i) invasion of hMSCs towards TNF α using a trans-well assay, (ii) chondrogenic differentiation of hMSCs in a 3D-micro-mass culture under hypoxic conditions, (iii) osteogenic differentiation of hMSCs, (iv) M-CSF/RANKL-mediated differentiation of isolated monocytes towards osteoclasts and (v) hypoxia-mediated target gene expression in non-differentiated, osteogenic and chondrogenic differentiated hMSCs.

Results: We demonstrate that Tofacitinib dose-dependently promotes the recruitment of hMSCs under hypoxia but inhibits recruitment of hMSCs under normoxia. With regard to the chondrogenic differentiation of hMSCs, we observed that Tofacitinib did not inhibit survival and at therapeutic relevant doses of 10-100nM. Moreover, Tofacitinib dose-dependently enhances osteogenic differentiation of hMSCs and reduces osteoclast survival and differentiation under hypoxic conditions. We show that Tofacitinib does not affect chondrogenic HIF target gene expression but increases HIF target gene expression in human osteogenic differentiated MSCs.

Conclusion: We conclude from our data, that Tofacitinib may influence bone healing by promotion MSC recruitment into the hypoxic microenvironment of the fracture gap, does not interfere with the cartilaginous phase of the soft callus phase of fracture healing process. We assume that Tofacitinib may promote bone

formation and reduce bone resorption which could in part explain the positive impact on bone erosions by Tofacitinib. Thus, we hypothesize that it will be unnecessary to stop this medication in case of fracture and suggest that positive effects on osteoporosis are likely.

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OP0075 PAR2 ACCELERATES OSTEOARTHRITIS-LIKE JOINT CHANGES IN A MURINE MODEL OF POST-TRAUMATIC OSTEOARTHRITIS

Kendal McCulloch¹, Carmen Huesa², Lynette Dunning¹, Rob Van't Hof³, John Lockhart¹, Carl Goodyear⁴. ¹University of West of Scotland, Institute of Biomedical and Environmental Health Research, Paisley, United Kingdom; ²University of Edinburgh, The Queen's Medical Research Institute, Edinburgh, United Kingdom; ³University of Liverpool, Institute of Ageing and Chronic Disease, Liverpool, United Kingdom; ⁴University of Glasgow, Institute of Infection, Immunity and Inflammation, Glasgow, United Kingdom

Background: Post-traumatic osteoarthritis (PTOA) is associated with articular cartilage damage and represents a major clinical challenge due to the poor regenerative capability of cartilage. We have recently developed a novel and robust dual injury murine model of PTOA, which combines destabilisation of the medial meniscus (DMM) and cartilage scratch¹. This model results in accelerated OA-like symptoms including enhanced osteophyte formation. Prior studies in the DMM model have demonstrated that absence of proteinase-activated receptor 2 (PAR2) in mice results in significant protection from early OA-like symptoms^{2,3}.

Objectives: To investigate if the absence of PAR2 confers protection in a dual injury PTOA murine model.

Methods: PTOA was induced in both male C57BL/6 wild-type (WT) and PAR2^{-/-} mice, via combined destabilisation of the medial meniscus and cartilage scratch (DCS). Twenty-eight days post-surgery, osteophyte formation and bone changes were monitored using microcomputed tomography. Dynamic weight bearing was assessed as an indirect measurement of pain at day 14.

Results: Evaluation of the presence and number of osteophytes revealed no significant differences between WT and PAR2^{-/-} mice at day 28. However, quantification of osteophytes revealed that PAR2^{-/-} mice had significantly smaller osteophytes (p=0.006) with less mineralised bone (p=0.003). Moreover, analysis of metaphyseal trabecular bone on the operated leg showed a significant decrease in bone volume/tissue volume (BV/TV) (p=0.025). Assessment of pain-related behaviour, using dynamic weight bearing at day 14, demonstrated that PAR2^{-/-} mice exerted less load on their front paws.

Conclusion: The findings in this study show that PAR2 plays a role in accelerated OA-like symptoms (i.e., osteophyte formation) in a dual injury model of OA, where both destabilisation of the medial meniscus and cartilage damage drive disease pathology. Furthermore, the loss of PAR2 decreases pain behaviour suggesting that PAR2 is involved in pain sensing. Taken together, these results support the future exploration of PAR2 as a therapeutic target for PTOA.

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OP0076 JAK-INHIBITORS TOFACITINIB AND BARICITINIB IMPROVE PATHOLOGICAL BONE LOSS IN VIVO

Susanne Adam¹, Nils Simon¹, Ulrike Steffen (Née Harre¹, Fabian Andes¹, Dorothea Müller¹, Stephan Culemann¹, Darja Andreev¹, Madeline Hahn¹, Carina Scholtyssek¹, Georg Schett¹, Gerhard Krönke¹, Silke Frey¹, Axel Hueber^{1,2}. ¹Friedrich-Alexander-University Erlangen-Nürnberg and Universitätsklinikum Erlangen, Department of Internal Medicine 3 – Rheumatology and Immunology, Erlangen, Germany; ²Sozialstiftung Bamberg, Rheumatology, Bamberg, Germany

Background: Targeting cytokines relevant to rheumatoid arthritis (RA) has proven efficient in clinical practice, but there is still demand for therapies that rebuild joint tissues which have been subjected to deterioration. Since many cytokines involved in RA rely on the intracellular janus kinase - signal transducer and activator of transcription (JAK-STAT) signaling pathway, targeting them presents itself as option. For this approach, JAK-inhibitors such as Tofacitinib and Baricitinib, targeting JAK1/JAK3 and JAK1/JAK2 respectively, seem favorable, as they have been approved for the treatment of RA¹. Moreover, preliminary data indicates an impact of JAK inhibition on local bone formation.

Objectives: To investigate the influence of JAK-inhibition on structural bone damage *in vivo* and its impact on osteoclast/osteoblast-mediated bone homeostasis *in vitro*.

Methods: *In vivo* analysis comprised unchallenged steady-state, the ovariectomy-induced mouse model of postmenopausal osteoporosis (OVX) and the serum-induced arthritis (SIA) mouse model. For steady-state analysis C57BL/6 (WT) mice obtained tofacitinib QD by oral gavage for 6 weeks. For OVX, WT mice received tofacitinib BID by oral gavage for 6 weeks. WT mice of the SIA model were fed tofacitinib or baricitinib BID for 14 days. Experimental readout included clinical parameters, ELISA (RANKL/OPG levels in serum), qPCR (mRNA expression in bone) and μ CT. For *in vitro* analysis, murine osteoclasts (OC) were analyzed with TRAP staining (osteoclastogenesis) and von Kossa staining (Resorptive capacity). Murine osteoblasts (OB), derived from mesenchymal stem cells (MSC) and calvariae were assessed with qPCR (differentiation) and Alizarin red staining (mineralization capacity).

Results: In steady-state conditions, JAK-inhibition by tofacitinib enhanced tibial trabecular bone density and decreased RANKL/OPG fraction in blood serum. These findings, and increased trabeculae numbers, were also applicable to spinal bone of tofacitinib-treated OVX mice. In SIA, both baricitinib and tofacitinib improved clinical symptoms and halted trabecular and cortical bone loss. *In vitro* OC-differentiation and function were not affected by JAK-inhibition. However, tofacitinib and baricitinib amplified OCN expression in MSC-derived OBs at day 1 after osteogenic induction, together with reduced Igf1 and elevated Dkk1 levels at day 7. Moreover, as a result of JAK-inhibition RANKL expression was decreased in calvaria-derived OBs. Accordingly, both MSC- and calvaria-derived OBs showed increased mineralization when treated with JAK-inhibitors.

Conclusion: Our results suggest that JAK-inhibition by tofacitinib and baricitinib causes increased mineralization by osteoblasts, resulting in enhanced bone density *in vivo*, in both unchallenged and pathological mouse models.

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OP0077 INNOVATIVE TRANSLATIONAL MODELS TO STUDY HUMAN SYNOVIAL PATHOLOGY: TARGET VALIDATION AND PRECLINICAL IMAGING

Irene DI Ceglie, Mathijs Broeren, Claire Waterborg, Daphne Dorst, R.M. Thurlings, Peter Laverman, Fons van de Loo, Peter van der Kraan, Peter van Lent, Marije Koenders. Radboud University Medical Center, Nijmegen, Netherlands

Background: Many experiments to study inflammation, hyperplasia, and fibrosis in the synovium have been performed in animal models of RA and OA. However, the predictive value of these models for the screening of potential drugs in RA is variable and for OA, none were sufficiently effective in clinical trials. Translational