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

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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

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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

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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

arthritis research with human cells is often performed in monolayer culture where the absence of extracellular matrix and other cell types results in alterations of cell functions and loss of phenotype.

Objectives: To improve the predictive value of preclinical arthritis research by developing and optimizing innovative translational models to study human synovial pathology in vitro and in vivo.

Methods: Synovial biopsies from RA patients were obtained during joint replacement surgery and processed for either (1) explant cultures, (2) 3D-synovial micromasses, (3) RA-SCID transplantation studies, and/or (4) a biobank for corresponding mRNA and IHC profiling. For explant culture, 3mm biopsies were cultured for 24hr w/o various inhibitors, and cytokine production was analyzed by Luminex. 3D-micromasses were generated from primary RA FLS and CD14+ PBMCs, stimulated for 3 weeks with 10 ng/ml TNF α or TGF β and analyzed by histology, IHC and QPCR. For target validation and preclinical imaging, 6mm biopsies were engrafted SC into SCID mice. Radionuclide- and fluorescently-labelled anti-CD64 antibodies were injected IV, and targeting was determined by biodistribution, μ SPECT/CT and IVIS imaging analysis.

Results: Our first assay with RA synovium explants demonstrated to be highly suitable to test the therapeutic efficacy of inhibitors for TNF α , TLR4, p38 and the JAK-pathway, resulting in significantly reduced production of proinflammatory mediators after 24hr of culture.

In contrast to the explant cultures, our 3D synovial micromasses could be followed for weeks. In this second translational model, lining formation was observed at day 7 and the micromasses could be stimulated to mimic RA- or OA-like features of synovial hyperplasia or fibrosis respectively. Long-term exposure to the RA-related cytokine TNF α lead to hyperplasia of the lining and an altered macrophage phenotype characterized by reduced CD163 expression. Conversely, the repair-related growth factor TGF β induced fibrosis-like changes in the micromass lining, a hallmark of OA. This was accompanied by an increased expression of *PLOD2*, *COL1A1* and α SMA.

Our third preclinical model, the RA synovium SCID mouse, was previously validated using adalimumab, secukinumab, and rituximab, and was now used to study CD64 as a potential marker to image synovitis. Gene expression of *FCGR1* (*CD64*) in synovial explants from RA patients was shown to correlate positively to gene expression of pro-inflammatory factors *IL1B*, *TNFA*, *IL8*, *S100A8*, *MCP1*, and with damage-associated genes *MMP2* and *MMP13*. Interestingly, dual-labelled ^{111}In -DTPA-IrDye800CW-anti-CD64 antibody showed high uptake in the synovial transplants of the SCID mice, and specifically visualized the subcutaneous synovial grafts by both μ SPECT/CT and IVIS imaging.

Conclusion: The development of these translational models allows us to bridge the gap between preclinical and clinical drug development research. Whereas our synovial explant assay is ideal for short-term interventions, and the RA-SCID mouse a great translational model for in vivo preclinical studies, donor variability and access to sufficient tissue may be challenging. In such cases, the 3D synovial micromass model may be an excellent alternative. Especially in combined setting, these 3 translational approaches will improve target validation and preclinical development of novel anti-rheumatic drugs.

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OP0078

LINKING LIPID MARKERS TO SYNOVIAL HYPERPLASIA AND VASCULARIZATION IN OSTEOARTHRITIS BY MALDI-MSI

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Background: Synovial inflammation or synovitis causes severe histological changes in the tissue affecting its normal functioning. These morphological changes are one of the most characteristic events in the OA pathology. Additionally, lipid species are recognized as important key factors in the development and regulation of inflammatory processes. Therefore, the lipid signature of the synovial membrane can provide new markers involved in the synovitis process undergoing the OA disease

Objectives: To define the specific lipid profile and distribution in human control and OA synovial membranes.

Methods: Human synovial membrane samples of OA patients (n=13) and controls (n=10) were compared by matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI). Tissue sections at 10 μ m thickness were mounted on conductive slides and coated with norharmane matrix (7mg/ml, 2:1 chloroform/methanol) for lipid extraction. MALDI images were acquired using a MALDI TOF-TOF instrument (Rapiflex MALDI TissueTyper, Bruker, Germany) in reflectron positive and negative ionization mode at a pixel size of 50 μ m. Multivariate statistical analysis was used to search for the lipids with the highest differences between OA and control synovial membranes. Then, lipid spatial distribution was investigated with FlexImaging 4.1 software. Identifications were based on tandem mass spectrometry analyses in comparison to LIPID MAPS Structure Database.

Figure 1. A) PCA-DA analysis separates control and OA samples according to lipid profile. B) Loading plot of PCA-DA analysis representing the lipid masses characteristic to OA (negative side) and control (positive side) samples given by MALDI-MSI. C) MALDI-MSI images showing the differences in the spatial distribution of two phosphatidylcholines in OA synovium.

Results: MALDI-MSI in combination with principal component analysis (PCA) and discriminant analysis (DA) revealed differential lipid profile between OA and control samples. Data acquired in positive ion mode showed a good separation of OA patients and controls (Figure 1A). OA tissues showed higher lipid content in the mass/charge (m/z) range 600-800, compared to controls (Figure 1B). Among identified lipid species, we found 35 phospholipids significantly differentially expressed between OA and controls, mainly classified in phosphatidylcholines (30%), phosphatidylethanolamines (26%), phosphatidylinositols (26%), phosphatidylserines (14%) and lysophosphatidylcholines (6%). Some of them showed a specific localization within tissue. For instance, the phosphatidylcholine (PC) m/z 734.55 showed a specific localization in the lining layer of hyperplastic OA synovium, whereas PC m/z 768.56 were located in the sublining layer characterized by high vascularity and inflammatory cell infiltrations (Figure 1C). The PCA-DA analysis also showed a higher presence of phosphatidylinositols (PIs) and phosphatidylethanolamines specifically in OA samples, whereas lysophosphatidylcholines were more abundant in control samples compared to OA. Most of these molecules, mainly PIs, were also spatially distributed in the lining layer of the OA synovium.

Conclusion: OA synovial tissues were characterized by a higher content of phospholipids, mainly PCs and PIs, compared to control synovial biopsies. Specific phospholipids related to morphological features of the synovial membrane have been described for the first time by MALDI-MSI. These molecules could have an important role in the synovitis associated with the pathogenesis of OA and constitute relevant molecular disease classifiers for the OA diagnosis.

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