**A3** TRANSLATIONAL PHARMACOLOGY STRATEGY TO EVALUATE MMP-13 INHIBITORS FOR THE TREATMENT OF OA; USE OF BIOMARKERS

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**Purpose:** Demonstrating efficacy of potential disease-modifying OA drugs (DMOAD) requires long treatment times and a large number of subjects, resulting in expensive clinical trials. An important risk of such studies is the absence of beneficial effect of the drug. Accurate prediction of the doses that would result in cartilage protection and joint preservation would help minimize this risk. Although dose may be extrapolated from pre-clinical studies, the lack of approved DMOADs obscures the clinical predictive capability of the OA animal models. Here we describe a translational pharmacology strategy using TIINE (a type II collagen neoepitope assay) to evaluate MMP-13 inhibitors for the treatment of OA.

**Methods:** The MMP-13 inhibitors belong to the class of compounds that bind the S\_3 pocket of the enzyme providing good potency (Ki 2–6 nM) and selectivity (>4000 fold over 15 other MMPs- ADAMTS-4 and -5, and TACE). TIINE was measured using a sandwich immunoassay and an LC- MS/MS assay specific for the 45-mer peptide. Rat medial meniscal tear (MMT) and dog partial medial meniscectomy (pMMx) were performed at Bolder BioPath as previously described. All studies were approved by the Institutional Animal Care and Use Committees.

**Results:** Studies in vitro showed that TIINE was generated by addition of IGFBP-3 to de-vitalized cartilage in a time- and concentration-dependent manner, which was inhibited by MMP-13 inhibitors. Because TIINE was also generated by addition of other MMPs, this biomarker is not specific for MMP-13 activity. In vivo, TIINE was upregulated about two fold in the urine from OA patients in contrast to age-matched controls. To understand the role of MMP-13 in the generation of such OA-associated TIINE, we analyzed TIINE modulation was evaluated in animal models of OA, and in response to MMP-13 selective inhibitors. In the rat MMT, TIINE was elevated in the synovial fluid (SF) from the operated knee, but not the contralateral knee, and it was inhibited in a time- and dose-dependent manner following oral administration of an MMP-13 inhibitor to a maximum of ~50%. Similar TIINE inhibition in the SF and urine was observed at the end of a 4-wk study (BID, prophylactic), and maximal inhibition correlated with cartilage protection as evaluated by histology. All animals and human subjects evaluated to date excrete some amount of TIINE in their urine (which is age-dependent). Single dose oral administration of MMP-13 inhibitor to skeletally-mature beagle dogs (naive) inhibited baseline levels of TIINE in a time- and dose-dependent manner up to ~50%. In the 4-wk dog pMMx model, MMP-13 inhibitors (BID, prophylactic) protected cartilage degeneration at exposures that inhibited TIINE ~45% at 2, 3 and 4 wks. These results suggest that doses that result in ~45% inhibition of TIINE in early clinical trials may protect cartilage degeneration and joint structure in Phase 2–3 studies. In previous clinical studies with broader spectrum MMP inhibitors, urinary TIINE was found to be inhibited in both human healthy volunteers and OA patients.

**Conclusions:** The proposed translational pharmacology strategy includes: (1) determine drug plasma and/or SF concentration required for maximum TIINE inhibition in urine of naive and/or OA animals, (2) establish correlation between TIINE inhibition and cartilage protection in animal models of OA, (3) evaluate TIINE inhibition in healthy volunteers and/or OA patients in early clinical trials, and (4) in POC studies, use the doses that resulted in sufficient inhibition of TIINE from the FIH studies and that was associated with cartilage protection in pre-clinical studies.

**A4** HYPOXIA-INDUCIBLE FACTOR 2A (HIF2A) CONTROLS SEQUENTIAL STEPS IN THE LATE STAGE OF ENDOCHONDRAL OSSIFICATION

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**Purpose:** The late stage of endochondral ossification including chondrocyte hypertrophy, cartilage matrix degradation, and vascular invasion are known to occur not only in physical growth, but also in cartilage destruction and osteophyte formation during osteoarthritis progression. Since the mechanism underlying these coordinated sequential steps remains an enigma, this study sought to identify the transcription factor and the related signals that control the stage.

**Methods:** A screen of transcription factors was performed using mouse chondrogenic ATDC5 cells and HeLa cells transfected with a luciferase-reporter construct containing a promoter of type X collagen (COL10), the marker for chondrocyte hypertrophy. The expression patterns were examined by immunohistochemistry of mouse growth plates and by real-time RT-PCR during insulin-induced differentiation of ATDC5 cells. Subcellular localization was examined by fluorescence microscope. Functional studies were performed using stable lines of ATDC5 cells with retroviral overexpression of HIF2A, and those with the dominant negative (DN) mutant or the small interfering RNA (siRNA). Transcriptional activity was determined by luciferase assay, and the specific binding between HIF2A protein and the identified region was verified by electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) assay. Physiological function of HIF2A was assessed by histological comparison of the skeletal phenotypes (endochondral ossification, HIF2A+/−) mice with those of the wild-type littermates (E16.5), since HIF2A+/− mice died in the early embryonic stage.

**Results:** Among over 100 transcription factors expressed in chondrocytes, hypoxia-inducible factor 2α (HIF2A), an α-unit member of the HIF family, most strongly stimulated the COL10 promoter activity. HIF2A was localized in the pre-hypertrophic and hypertrophic zones of the growth plate, and the expression increased during differentiation of ATDC5 cells in association not only with COL10 expression, but also with expressions of matrix metalloproteinase 13 (MMP13) and vascular endothelial growth factor (VEGF), crucial factors for matrix degradation and vascular invasion respectively. Expressions of COL10, MMP13 and VEGF, as well as ALP and Alizarin red stainings, were enhanced by the HIF2A overexpression in ATDC5 cells, but were suppressed by the DN mutant or the gene silencing through siRNA. The promoter activities of the three genes were enhanced by the HIF2A overexpression. Deletion and mutation analyses identified the respective responsive elements, which were confirmed to show specific binding with HIF2A by EMSA and ChIP assay. The HIF family is known to work as a heterodimer of α-unit and β-unit members. Our search for the β-unit partner found that aryl hydrocarbon receptor nuclear translocator-like (ARNTL, also known as BMAL1), which was co-localized with HIF2A in the nucleus of differentiated chondrocytes, most strongly enhanced the transcription of the three promoters by HIF2A, and was necessary for the specific bindings with the responsive elements and HIF2A. Finally, the HIF2A+/− mice were confirmed to exhibit dwarfism with impairment of the late stage of endochondral ossification in the growth plate cartilage.

**Conclusions:** HIF2A is the crucial transcription factor that controls the late stage of endochondral ossification through direct transactivation of COL10, MMP13 and VEGF. Elucidation of the signals related to HIF2A will lead to further understanding of the molecular background of osteoarthritis.

**A5** AGE-RELATED CHANGES IN CHONDROCYTE DIFFERENTIATION MAKES CARTILAGE PRONE TO OA DEVELOPMENT

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**Purpose:** Chondrocytes in articular cartilage developing OA show deviant behavior. They seem to display a recapitulation of chondrocyte differentiation similar to chondrocyte hypertrophy in the growth plate. In the growth plate, Indian Hedgehog (Ihh) blocks terminal differentiation by keeping the chondrocytes in a pre-hypertrophic state. Loss of Ihh expression releases this block and allows the chondrocytes to undergo further differentiation. This occurs with the hypothalamic gene, alizarin red staining is seen not only in the hypertrophic zone, but also in the prehypertrophic and perichondral regions. Our search for the Ihh expression using immunohistochemistry in cartilage during experimental OA induced in C57Bl/6 mice by either injection of collagenase (14, 21 and 42 days) or by destabilization of the medial meniscus (DMM model) (8 weeks). Ihh expression in spontaneous OA was analyzed in STR/or mice aged 8 weeks and 3, 6, 9 and 12 months. Results: In the former, Ihh expression was expressed abundant Ihh in chondrocytes of the articular cartilage. With age the number of Ihh immunopositive cells rapidly declined. The number of Ihh positive cells declined 72% by 6 months of age compared to 3-month-old mice and declined even further after that. In lateral tibial cartilage the response was similar, but with a