Fibroblast growth factor-18 (FGF18) plays an important role during skeletal development in mammals and appears to be a trophic factor for chondrocytes of hyaline cartilage. We have previously shown that Fgf18 and the genes for two of its receptors are expressed in adult human articular cartilage. Addition of FGF18 to the culture medium of primary articular chondrocytes increased both the proliferation of these cells and their production of extracellular matrix. Chondrocyte proliferation and matrix deposition were also observed at a number of sites in vivo following local or systemic delivery of FGF18 in a variety of species. To evaluate whether FGF18 could generate chondral tissue and reverse cartilage degeneration in a setting of osteoarthritis (OA), OA was induced by creating a meniscal tear in the knee joint of rats. FGF18 was dissolved in a hyaluronan carrier and was applied to the operated knee by intra-articular injection. The repair of cartilage degeneration was evaluated 3 weeks later.

**Materials and Methods:** The medial collateral ligament of each rat (n=10 rats per group) was transected and the medial meniscus was cut through the full thickness to simulate a complete tear. Three weeks after surgery, rats received intra-articular injections of either vehicle (0.5% hyaluronan) or vehicle containing E. coli-derived recombinant human FGF18 (0.1, 1.0, or 5.0 µg) twice per week for three weeks. Four days after the last injection, the knee joints were harvested, collected into buffered formalin, decalcified, and embedded in paraffin for histology. Frontal sections of the knee joints were stained with toluidine blue to assess formation of chondral tissue. An image of the tibial plateau of each knee was captured using an Optimas image analysis system. Multiple sections of the right knee were analyzed microscopically and scored subjectively for cartilage degeneration (chondrocyte/matrix loss and fibrillation) and chondrophyte formation. Strict attention to zones (outside, middle, and inside thirds of the medial tibial plateau) was adhered to and summed to reflect total severity of tibial degeneration.

Micrometer measurements of the total extent of the tibial plateau affected by degeneration, width of tibial lesions that extended >50% of cartilage thickness (Tibial Cartilage Degeneration Width), lesion depth (Depth Ratio), thickness of the medial tibial cartilage to the tidemark, and chondrophyte size and number were assessed. Statistical analysis of histopathologic parameters was done by comparing group means using analysis of variance. All injections and scoring were performed by investigators blinded to the treatment groups.

**Results:** In this model, damage to the meniscus induces progressive cartilage degeneration and osteophyte formation that mimic the changes that occur in spontaneous osteoarthritis. The degeneration of cartilage is most severe on the outer two-thirds of the tibial plateau and reaches maximal levels at 3 weeks following the meniscal damage. FGF18 was administered from 3 to 6 weeks following surgery to determine if it could induce repair of the damaged cartilage. FGF18 induced a dose-dependent increase in cartilage hypertrophy and overgrowth of new cartilage around the damaged areas as well as normal cartilage in the lateral compartment. These results are shown in Figure 1. The highest dose of FGF18 (5 µg) resulted in a 57% decrease (p<0.05) in cartilage degeneration scores for the outer 1/3 of the tibial plateau, a 57% reduction (p<0.05) in the width of significant tibial cartilage degeneration, and a 46% decrease (p<0.05) in depth ratio for any matrix change as a result of filling of the cartilage defects with repair tissue. In addition, FGF18 produced dose-dependent increases in medial tibial cartilage thickness, from 243 ± 21 to 319 ± 77 µm (mean ± SD, p<0.05) in rats treated with vehicle or 5.0 µg of FGF18, respectively. The morphology of the repair tissue ranged from fibrous with proteoglycan deposition to fibrocartilage. Repair tissue appeared to originate from the marginal zone areas and extended across the degraded and sometimes intact surfaces. In nearly all areas, repair tissue appeared to integrate well with the margins of the remaining normal cartilage. Although the morphology of the repair tissue was different from hyaline cartilage, it appeared to effectively fill the defect and there were virtually no degenerative changes. In contrast to the FGF18-treated animals, rats treated with vehicle alone showed no signs of cartilage repair except in rare cases where erosion of the subchondral bone permitted influx of bone marrow stem cells.

In addition to FGF18-mediated chondrogenesis, other changes were noted in the FGF18-treated joints. The medial tibia chondrophyte measurement at the 1 or 5 µg doses of FGF18 was increased 50% (p<0.05), and the subchondral bone scores, indicative of bone resorption, were increased by 31% the highest dose of FGF18 (p<0.05). A dose-dependent increase in synovial hyperplasia was also noted and animals that received the highest dose of FGF18 showed mild joint swelling beginning at the second week of treatment.

**Discussion:** We have used the rat meniscal tear OA model to demonstrate that FGF18 delivered in a hyaluronan carrier can elicit significant repair of damaged cartilage. The newly-generated cartilage induced by the highest dose of FGF18 resulted in a 31% increase in the medial tibial cartilage thickness and reduced the cartilage lesion degeneration scores in the outer 1/3 of the medial tibial plateau by approximately 50%. This newly proliferated cartilage appeared to be well-integrated with the normal cartilage thus providing a load-bearing tissue that could withstand any abnormal biomechanics and stresses imposed by damage to the meniscus. Although higher doses of FGF18 also appeared to increase some inflammatory events such as synovial hyperplasia and bone resorption, it is anticipated that modification of the dosing regime could reduce these effects. The significant cartilage repair induced by FGF18 in this OA model suggests that this factor may have utility in the treatment of cartilage damaged by osteoarthritis or injury.

**Conclusion:** These data demonstrate that local delivery of FGF18 in a hyaluronan carrier can increase cartilage formation and can reduce cartilage degeneration scores in a rat model of osteoarthritis.

*ZymoGenetics, Inc., Seattle, WA
**Bolder BioPATH, Inc., Boulder, CO

Paper No: 0199