Supplementary Pathological Evaluation

This evaluation summarizes our histopathological analyses on the tissue slides of *Nfat1*$^{-/-}$ and wild type (WT) mice. It is our unanimous opinion that observed cartilage cells in and around affected articular cartilage of *Nfat1*$^{-/-}$ mice do not display diagnostic characteristics of neoplasia or cartilaginous tumors. However, these *Nfat1*$^{-/-}$ joints do show osteoarthritic changes. Therefore, our pathological diagnosis on the phenotype of the *Nfat1*$^{-/-}$ mouse joints is osteoarthritis (OA) or osteoarthritic joint degeneration. Our diagnosis is based on the following observations.

(1) We have analyzed the age-dependent changes of *Nfat1*$^{-/-}$ mouse joints and found that a loss of proteoglycans in articular cartilage is already present at 2 months of age, prior to the formation of periarticular cartilage, suggesting that the formation of periarticular cartilage and osteophytes in *Nfat1*$^{-/-}$ mice is a secondary reparative reaction to the dysfunctional articular cartilage.

(2) Most of the characteristics of human OA, such as loss of articular cartilage proteoglycans, roughening of articular surface, formation of periarticular chondro-osteophytes, focal loss of articular cartilage with exposure of thickened subchondral bone (eburnation), and narrowing of joint space with fibrous joint fusion, are observed in *Nfat1*$^{-/-}$ mouse joints. The overall picture fits a diagnosis of OA.

(3) Cartilage differentiation as seen in *Nfat1*$^{-/-}$ mouse joints during osteophyte development first occurs in the synovium adjacent to the edges of articular cartilage, where later chondro-osteophytes and osteophytes form through endochondral ossification. Osteolytic lesions are not observed in *Nfat1*$^{-/-}$ joints. In contrast, chondrosarcoma is commonly osteolytic at sites of origin, usually located in the metaphysis. Osteochondroma (the most common benign cartilaginous tumor) and enchondroma (a usually benign cartilaginous tumor) also commonly arise in the metaphyseal region of long bones and not in synovium near articular cartilage.

(4) Neoplastic cellular changes are not observed in *Nfat1*$^{-/-}$ mouse joints. Although the development of chondrophytes and chondro-osteophytes is exuberant and almost tumor-like in some of the hip joints, malignant-looking cartilage cells with large single or multiple bulging nuclei and hyperchromatism are not seen in and around *Nfat1*$^{-/-}$ mouse joints.

(5) Dr. K. Krishnan Unni, Professor Emeritus of Pathology at the Mayo Clinic and a world authority in bone tumors, has also reviewed the same tissue slides of *Nfat1*$^{-/-}$ and WT mice that we examined. Dr. Unni described that *Nfat1*$^{-/-}$ mouse joints displayed fissuring in articular cartilage, narrowing of the joint space, and osteophytes in which cells were clearly not neoplastic. He diagnosed the phenotype of *Nfat1*$^{-/-}$ mouse joints as degenerative joint disease, not cartilaginous tumors. Dr. Unni's written evaluation is available upon request.

Pathological evaluators at the University of Kansas Medical Center:

H. Clarke Anderson, M.D.
Professor of Pathology
Harrington Professor of Orthopedic Research
handerso@kumc.edu

Ossama Tawfik, M.D., Ph.D.
Professor of Pathology
Director of Anatomy and Surgical Pathology
otawfik@kumc.edu

David M. Pinson, D.V.M., Ph.D.
Diplomate, American College of Veterinary Pathology
Professor of Pathology, Director of Laboratory Animal Resources
dpinson@kumc.edu