B. Stride frequency also trended towards normalization post IA BoNT B, approaching statistical significance (p = 0.0624). Evoked pain behaviors improved following IA BoNT B, but did not reach statistical significance. No weakness of the hindlimb was noted following IA BoNT B (grasp p = 0.7872 clinging p = 0.5433).

**Conclusions:** Chronic degenerative arthritis pain can be quantified in a murine model using visual and computerized gait analysis, and joint tenderness scores. Visual and computerized gait analysis both showed a significant impairment in gait in arthritic mice, improving after IA BoNT B suggesting a substantial analgesic effect. Evoked pain responses increased overall with arthritis, decreasing with IA BoNT B, but not reaching statistical significance. Further studies are underway to expand on these results.

These findings support the hypothesis that chronic arthritis pain may be amplified by neuropeptide release in the periphery due to efferent neurotransmitter signal. Interruption of neuropeptide release by IA BoNT B appeared to decrease pain responses in the joint and improve gait abnormalities. IA BoNT B was safe: no increased joint inflammation nor weakness of limb was noted. These results further support investigation of this novel approach to treatment of arthritis pain with IA neurotoxin.

**498**

**A T-CELL ASSOCIATED CYTOKINE, INTERLEUKIN-17, PROMOTES AN INFLAMMATORY PHENOTYPE IN DEGENERATIVE HUMAN INTERVERTEBRAL DISC CELLS**

M.A. Gabr1, L.A. Setton2, S.M. Sinclair2, L. Jing2, C.R. Brown1, W.J. Richardson3, J. Chen2


**Purpose:** Interleukin-17 (IL17) is a novel T helper cell-associated cytokine recently found to be present at high levels in both herniated and degenerate tissues of the intervertebral disc (IVD). IL17 and other Th17-related cytokines are known regulators of inflammation in multiple autoimmune diseases, suggesting their potential involvement in regulating IVD pathology. The objective of this study is to determine the effects of IL17 and a potent co-stimulant, interferon (IFNγ) on human cells from degenerated IVD sources. An ability for IL17 with IFNγ to promote inflammatory cytokine and mediator expression was assessed in IVD cells and compared to effects of another potent pro-inflammatory cytokine, TNFα.

**Methods:** Human IVD cells were isolated from to-be discarded tissues from patients undergoing surgery for degenerative IVD pathology (n=4). Cells of the anulus fibrosus (AF) region were plated at 25,000 cells per well (96-well plates, n=4 wells per group) and overlaid with 150 μL of medium overnight (5% CO2, 37°C). Media was then replaced with new media supplemented with one of the following cytokine concentrations: media alone (control), TNFα (25 ng/mL), IL17 (10 ng/mL), IFNγ (200 U/mL) or both IL17 and IFNγ. After 72 hours, the supernatant was evaluated for release of nitric oxide (NOx, Griess reaction) and prostaglandin E2 (PGE2, ELISA). One-way ANOVA and post-hoc Dunn’s analysis evaluated differences amongst treatment groups for NOx and PGE2 release (p=0.05).

**Results:** Stimulation of AF cells with IL17 or IFNγ alone did change NOx levels from control values; however, a significant variability in NOx responses was observed across human subjects (Figure 1 left). In the presence of IFNγ costimulant, IL17 induced average 2-fold increase in NOx production above that of IL17 alone (IL17: average NOx = 22 μM, IL17 + IFNγ: NOx = 45 μM, Figure 1 left). In contrast, stimulation with IL17 alone led to a significant elevation in PGE2 levels in all 4 samples (average 14-fold increase, Figure 1 right). As for the NOx result, co-stimulation with IL17 and IFNγ led to a large increase in PGE2 release, more than 24-fold over control values (Figure 1 right, IL17 + IFNγ: PGE2 release = 1.36 ng/ml; Control PGE2 release = 0.06 ng/ml). In general, the induction of NOx and PGE2 release by IL17 and IFNγ co-stimulation was significantly larger than that due to TNFα stimulation, for which few changes in NOx and a 10-fold increase in PGE2 was observed.

**Conclusions:** The results of this study demonstrate that degenerative human IVD cells may respond to IL17 stimulation with increased production of the inflammatory mediators, PGE2 and NOx, with a more robust effect observed in the presence of IFNγ. Degenerative and herniated IVD explants are known to contain IFNγ, and IFNγ is known to act synergistically with IL17 to promote release of inflammatory mediators in other cell types. This work demonstrates that the responsiveness of IVD cells to IL17 and IFNγ is consistent with other cell types, and suggests a potential role for these cytokines in contributing to IVD pathology.

**499**

**SUSTAINED RELEASE OF SFLT01 IS NECESSARY AND FEASIBLE FOR PROLONGED INTRAARTICULAR DELIVERY TO THE KNEE TO NEUTRALIZE VEGF LOCALLY**

D. Li1, D. Ralphs1, M. O’Callaghan1, N. Moran1, J. Serriello1, E. Johnston2, D. Gianolio2, K. Albee2, J. Kingsbury1, J. Bird1, R. Simler1, W. Brondyk1, K. Culm-Larsen1, K. Barranco1, B. Greene1, G. Matthews1

1Genzyme Corp., Framingham, MA; 2Genzyme Corp., Waltham, MA

**Purpose:** We hypothesize that osteoarthritides (OA) pain-associated bone marrow lesions, effusion, and synovitis are driven pathophysiologically by VEGF mediated increases in vascular permeability, and that local inhibition of VEGF will alleviate OA pain by decreasing incidence and severity of these abnormalities. Our previous work demonstrated that VEGF inhibition using virally delivered soluble Flt receptor (sFlt) decreased synovitis and pain marker expression in a rabbit OA model. In an effort to move...