

### Use of Serum Biomarkers to Evaluate OA Treatment Using Gene Transfer

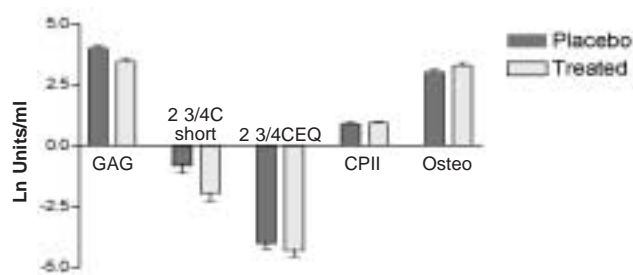
**Summary:** As part of a larger study (reported in the 1999 Lab Report), this work evaluated the use of serum biomarkers to monitor therapeutic improvement seen following over-expression of the potentially anti-arthritic gene sequence for equine interleukin-1 receptor antagonist (IL-1Ra) in horses with experimental osteoarthritis (OA). Significant benefits were seen in parameters of clinical pain and disease activity, as well as in histologic changes observed within joint tissues. Serum biomarker levels suggest that decreasing GAG release and less type I collagen degradation may be one way that IL-1Ra therapy preserves joint tissue health. Furthermore, this study demonstrated that serum markers may be helpful in monitoring improvement in OA following therapeutic intervention.

The objective of this study, done by Dr. David Frisbie, Dr. Clark Billingham, Megan Knowlton, Drs. Steven Ghivizzani, Paul Robbins, Gayle Trotter, Chris Evans and Wayne McIlwraith, was to evaluate the use of serum biomarkers to monitor therapeutic improvement seen following over-expression of the potentially anti-arthritic gene sequence for equine interleukin-1 receptor antagonist (IL-1Ra) in horses with experimental osteoarthritis (OA). This study was part of a larger experiment evaluating gene therapy for the treatment of OA which was reported in the 1999 Orthopaedic Laboratory Report.

Using the published gene sequence for equine IL-1Ra (previously developed by Dr. Rick Howard in this laboratory), an E1/E3 deleted adenoviral vector (Ad-EqIL-1Ra) was constructed that was capable of equine IL-1Ra transgene expression. In accordance with institutional Animal Care and Use Committee approval, osteochondral fragments were created in one intercarpal joint of 16 horses. On day 14 post-fragment creation, one randomly chosen fragmented joint of 8 horses was directly administered the Ad-EqIL-1Ra vector while the remaining joints received placebo treatment. The horses were exercised 5 days per week for the remaining 56 days of the study. Throughout the study, the researchers collected serum every 7 days; on day 70 post-fragment creation, the horses were clinically re-evaluated and then euthanized. Gross pathologic changes were documented and synovial membrane and articular cartilage collected for histologic analysis. Serum was analyzed for biomarkers related to joint tissues at each time point and a statistical comparison made at day 70.

Upregulation of IL-1Ra expression was demonstrated and was associated with significant improvement in gross articular cartilage lesions, clinical parameters of pain and disease activity, as well as beneficial effects in histologic parameters measured from synovial membrane and articular cartilage. Significantly smaller amounts of collagen fragments (COL2-

3/4C<sub>short</sub>) and glycosaminoglycans (GAG) were detected in serum from IL-1Ra treated horses compared to placebo treated horses at the termination of project. No significant differences were detected in bone formation (osteocalcin), type II collagen synthesis (CPII) or type II collagen degradation (234CEQ) in a similar comparison (Figure 1).



Results of this study suggest significant benefits were seen in parameters of clinical pain and disease activity, as well as in histologic changes observed within joint tissues. Serum biomarker levels suggest that decreasing GAG release and less type I collagen degradation may be one way that IL-1Ra therapy preserves joint tissue health. Furthermore, this study demonstrated that serum markers may be helpful in monitoring improvement in OA following therapeutic intervention.

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#### Publications

Frisbie DD, McIlwraith CW. "Evaluation of gene therapy as a treatment for equine traumatic arthritis. A species with clinical disease." *Clin Orthop Op Rel Res.* 2000. S795: 5273-5287.

Frisbie DD, Ghivizzani SC, Robbins PD, Evans CH, McIlwraith CW. "Treatment of experimental osteoarthritis by an in-vivo delivery of the equine interleukin-1 receptor antagonist gene." *Gene Therapy.* 2002. 9: 12-20.