

### ***Evaluation of Interleukin-1 Receptor Antagonist Delivered Using an Adeno Associated Viral Vector for Treatment of Experimentally Created Osteoarthritis in the Horse***

This project is designed to analyze the effects of gene-transfer delivered interleukin-1 receptor antagonist protein (IL-1Ra) in the treatment of Osteoarthritis (OA). OA has been cited as the most economically important skeletal disease in horses. Therapeutic intervention of OA is hindered in part by the inability to target therapeutic agents directly to the joint. Gene therapy provides a possible answer to this problem as a single intra-articular (IA) injection can result in local production of a specific therapeutic protein within diseased joint(s) for a prolonged period of time. It is hoped that using a vector other than a human engineered adenovirus to deliver IL-1Ra to the synovial space will reduce damage to joint tissues induced by traumatic and inflammatory model OA.

Previously an engineered human adenoviral vector capable of expressing the equine IL-1Ra gene (Ad-EqIL-1Ra) was evaluated in vitro to confirm its ability to infect equine synovial fibroblasts and express biologically active equine IL-1Ra. In vivo studies demonstrated that IA administration of Ad-EqIL-1Ra improved clinical parameters of pain and disease activity. Gene transfer reduced certain aspects of synovitis while protecting articular cartilage from damage. However, problems with this vector technology include: inflammatory reactions to vector capsid and proteins produced by the vector (related to its virus function), the ability to readminister the vector, and its tropism for human cells. In attempts to alleviate these issues, this project

proposed to construct an adeno-associated virus (AAV) expression vector that uses the equine IL-1Ra gene sequence to drive IL-1Ra protein expression (AAV-eqIL-1Ra).

AAV differs from other viruses in that it is naturally defective; it requires cotransfection with a helper virus for proliferate infection to occur. Although the majority of the population has been exposed to AAV, no human disease has yet to be associated with AAV infection. However, AAV has the ability to infect cells originating from different species, tissue types, in both dividing and non-dividing cells in vitro and in vivo. AAV is also capable of integrating into the host genome in a site-specific manner, providing the potential for long-term gene expression in vivo.

The AAV-eqIL-1Ra vector will be tested in vitro by quantifying equine IL-1Ra levels from equine synoviocytes transduced by AAV-eqIL-1Ra. In vivo studies will establish the level and duration of expression or equine IL-1Ra produced by the vector following IA administration. Therapeutic effects of AAV delivered IL-1Ra will be evaluated on a traumatic model of OA in horses.

Currently, the AAV expression vector is being constructed by Lori K. Hermann, working with Dr. Frisbie and Andrea Hume.

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