

## Category E explanation:

### Unrelieved pain due to osteoarthritis.

The use of analgesics are an effective approach to preventing OA pain in the rabbit ACLT model and analgesics will be used pre-operatively, b.i.d. for 72 hrs post-operatively, and as needed beyond a post-operative period of 72 hours for studies that will not examine the effect of our hydrogel on OA pain. Notably, however, we are interested in examining whether our hydrogel and PGRN-derivative are capable of alleviating pain associated with OA and ACLT is an accepted model of persistent joint pain [1]. The limitations of animal models of pain, particularly with regard to evaluations of analgesic efficiency, are familiar to investigators [2,3,4,5]. However, the necessity of controlled conditions that allow for examination of pain indicators in conjunction with characterization of cellular and molecular changes brought on by treatment (offered by an animal model and unachievable in human subjects at this stage) is integral to defining the potential of our hydrogel and molecules of interest in therapeutic applications.

For animals in pain studies, the initial post-surgical period of pain will be attenuated with post-operative (b.i.d. every 8-12 hours for 72hours post-op) administration of buprenorphine. Application of opioid analgesia beyond this 72 hour period is not appropriate in our pain studies. The use of analgesic beyond the 72-hour post-surgical period would not allow us to determine whether any observed difference in pain associated behaviors among treatment groups was due to the influence of the hydrogel and/or the Atsttrin/PGRN loaded hydrogel or due to the influence of an accepted analgesic. NSAIDs cannot be used for these animals due to interference with inflammation and study goals.

Previous studies have demonstrated that administration of analgesics preceding pain analyses is associated with changes in gait analysis parameters in human OA patients [6-10]. Animal models of OA pain also reflect changes in hyperalgesia, gait and/or activity level following administration of substances with analgesic activity [3, 5, 10-16]. Partial reversal of arthritis-associated gait abnormalities following administration of clinical analgesics and other anti-nociceptive substances suggests that altered gait is indicative of pain in inflammatory and neuropathic disease models and that reversal of gait abnormalities may indicate analgesic effect. The sensitivity of pain indicators to reversal via intervention with analgesics has been observed at multiple timepoints, ranging from immediately post-induction to 28 days post-induction [11,12,14,15]. Coulthard and colleagues have previously demonstrated that a single dose of buprenorphine, administered before spontaneous ambulation, was able to reverse velocity, stride length, and single stance time abnormalities, which had emerged as early as day 10 following induction of an adjuvant-induced arthritic rat model [16]. With more specific regard to surgically induced models of

arthritis, analgesics have been demonstrated to have a therapeutic effect upon joint pain when administered 4 days after surgery[17]. Further, tactile allodynia and weight bearing changes associated with surgically induced OA can persist and can be attenuated with administration of pain killers up to, at least, 21 days post-surgery [12].

Although the majority of literature regarding the effect of analgesic upon indicators of arthritic pain comes from rodent models, available literature does establish that analgesics appropriate for use in the rabbit are associated with at least partial reduction in indicators of spontaneous/continuing pain. Accordingly, the use of analgesics beyond the period of post-operative pain would introduce an insurmountable confounding factor in evaluating the effect of our treatments on arthritic pain.

[1] Committee on Recognition and Alleviation of Pain in Laboratory Animals, Institute for Laboratory Animal Research, Division on Earth and Life Studies, and National Research Council. "Appendix A." In Recognition and Alleviation of Pain in Laboratory Animals, 143-55. National Academies Press, 2009.

[2] Chapman, C. R., K. L. Casey, R. Dubner, K. M. Foley, R. H. Gracely, and A. E. Reading. "Pain Measurement: An Overview." *Pain* 22, no. 1 (1985): 1-31. doi:10.1016/0304-3959(85)90145-9.

[3] Suokas, A.k., D.r. Sagar, P.i. Mapp, V. Chapman, and D.a. Walsh. "Design, Study Quality and Evidence of Analgesic Efficacy in Studies of Drugs in Models of OA Pain: A Systematic Review and a Meta-analysis." *Osteoarthritis and Cartilage* 22, no. 9 (2014): 1207-223. doi:10.1016/j.joca.2014.06.015.

[4] Mogil, Jeffrey S., Karen D. Davis, and Stuart W. Derbyshire. "The Necessity of Animal Models in Pain Research." *Pain* 151, no. 1 (2010): 12-17. doi:10.1016/j.pain.2010.07.015.

[5] Cortright, Daniel N., David J. Matson, and Daniel C. Broom. "New Frontiers in Assessing Pain and Analgesia in Laboratory Animals." *Expert Opinion on Drug Discovery* 3, no. 9 (2008): 1099-108. doi:10.1517/17460441.3.9.1099.

[6] Schnitzer, Thomas J., Jovan M. Popovich, Gunnar B. J. Andersson, and Thomas P. Andriacchi. "Effect of Piroxicam on Gait in Patients with Osteoarthritis of the Knee." *Arthritis & Rheumatism* 36, no. 9 (1993): 1207-213. doi:10.1002/art.1780360905.

[7] Shrader, M. Wade, Louis F. Draganich, Lawrence A. Pottenger, and Gary A. Piotrowski. "Effects of Knee Pain Relief in Osteoarthritis on Gait and Stair-Stepping." *Clinical Orthopaedics and Related Research* 421 (2004): 188-93. doi:10.1097/01.blo.0000119248.70353.a5.

[8] Henriksen, Marius, Erik B. Simonsen, Tine Alkjær, Hans Lund, Thomas Graven-Nielsen, Bente Danneskiold-Samsøe, and Henning Bliddal. "Increased Joint Loads during Walking – A Consequence of Pain Relief in Knee Osteoarthritis." *The Knee* 13, no. 6 (2006): 445-50. doi:10.1016/j.knee.2006.08.005.

[9] Boyer, Katherine A., Martin S. Angst, Jessica Asay, Nicholas J. Giori, and Thomas P. Andriacchi. "Sensitivity of Gait Parameters to the Effects of Anti-

- inflammatory and Opioid Treatments in Knee Osteoarthritis Patients." *Journal of Orthopaedic Research* 30, no. 7 (2011): 1118-124. doi:10.1002/jor.22037
- [10] Marcum, Zachary A., Hanzi Lena Zhan, Subashan Perera, Charity G. Moore, G. Kelley Fitzgerald, and Debra K. Weiner. "Correlates of Gait Speed in Advanced Knee Osteoarthritis." *Pain Med Pain Medicine* 15, no. 8 (2014): 1334-342. doi:10.1111/pme.12478.
- [11] Fernihough, Janet, Clive Gentry, Marzia Malcangio, Alyson Fox, John Rediske, Theodore Pellas, Bruce Kidd, Stuart Bevan, and Janet Winter. "Pain Related Behaviour in Two Models of Osteoarthritis in the Rat Knee." *Pain* 112, no. 1 (2004): 83-93. doi:10.1016/j.pain.2004.08.004.
- [12] Bove, S.e., K.d. Laemont, R.m. Brooker, M.n. Osborn, B.m. Sanchez, R.e. Guzman, K.e. Hook, P.l. Juneau, J.r. Connor, and K.s. Kilgore. "Surgically Induced Osteoarthritis in the Rat Results in the Development of Both Osteoarthritis-like Joint Pain and Secondary Hyperalgesia." *Osteoarthritis and Cartilage* 14, no. 10 (2006): 1041-048. doi:10.1016/j.joca.2006.05.001
- [13] Gotoh, Mari, Aya Nagano, Ryoko Tsukahara, Hiromu Murofushi, Toshiro Morohoshi, Kazuyuki Otsuka, and Kimiko Murakami-Murofushi. "Cyclic Phosphatidic Acid Relieves Osteoarthritis Symptoms." *Molecular Pain Mol Pain* 10, no. 1 (2014): 52. doi:10.1186/1744-8069-10-52.
- [14] Hashizume, Misato, Nobuo Koike, Hiroto Yoshida, Miho Suzuki, and Masahiko Mihara. "High Molecular Weight Hyaluronic Acid Relieved Joint Pain and Prevented the Progression of Cartilage Degeneration in a Rabbit Osteoarthritis Model after Onset of Arthritis." *Mod Rheumatol Modern Rheumatology* 20, no. 5 (2010): 432-38. doi:10.1007/s10165-010-0299-1.
- [15] Mihara, M., S. Higo, Y. Uchiyama, K. Tanabe, and K. Saito. "Different Effects of High Molecular Weight Sodium Hyaluronate and NSAID on the Progression of the Cartilage Degeneration in Rabbit OA Model." *Osteoarthritis and Cartilage* 15, no. 5 (2007): 543-49. doi:10.1016/j.joca.2006.11.001.
- [16] Coulthard, Paul, Barbara J. Pleuvry, Mike Brewster, Kevin L. Wilson, and Tatiana V. Macfarlane. "Gait Analysis as an Objective Measure in a Chronic Pain Model." *Journal of Neuroscience Methods* 116, no. 2 (2002): 197-213. doi:10.1016/s0165-0270(02)00042-0.
- [17] Castro, R.r., F.q. Cunha, F.s. Silva, and F.a.c. Rocha. "A Quantitative Approach to Measure Joint Pain in Experimental Osteoarthritis evidence of a Role for Nitric Oxide." *Osteoarthritis and Cartilage* 14, no. 8 (2006): 769-76. doi:10.1016/j.joca.2006.01.013.

Pain associated with the operation procedure and PTOA development will be attenuated with analgesics. For studies focused on the onset and development of PTOA, pain associated with OA will be attenuated with analgesics. For studies concerned with the effect of Atsttrin, PGRN and the hydrogel upon OA pain, analgesics will be provided pre and post-operatively to attenuate surgical pain. Pain associated with OA development will be monitored and early experimental and/or humane endpoints will be implemented as necessary, under direction of veterinary staff, to minimize suffering.