Anti-Nerve Growth Factor Monoclonal Antibodies for Chronic Pain Control in Cats and Dogs:

A Review of Clinical Outcomes

PEER REVIEWED

Chronic pain management is an area of major clinical importance. Multiple prevalent conditions in veterinary species are associated with pain and disability and the limitations of existing therapeutic options, especially in cats, have resulted in significant unmet medical need. In human Phase III clinical studies, monoclonal antibody (mAb) therapies targeting the hormone nerve growth factor (NGF) have been shown to be highly effective in managing chronic pain. We review here outcomes from pre-clinical and clinical studies of fully caninised and fully felinised anti-NGF mAbs, which support the further development of these therapies for chronic pain management in their respective species.

Degenerative Joint Disease and Current Approved Treatments in Dogs and Cats

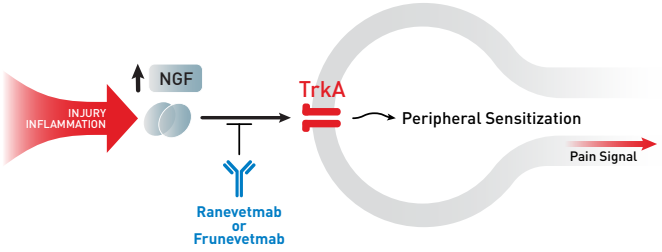
In veterinary medicine, the mainstay of drug therapy for the alleviation of clinical signs associated with degenerative joint disease (DJD, including osteoarthritis) -associated pain in dogs and cats are non-steroidal anti-inflammatory drugs (NSAIDs). This may be partly due to the fact that there are no other classes of drug approved by the Food and Drug Administration's Center for Veterinary Medicine (FDA CVM) for the control of DJD-associated pain in dogs. In cats, the NSAID meloxicam is currently approved in Europe for use in treating chronic pain, but has not been approved for this use in the United States. There are no other approved medications for the long-term treatment of chronic pain in the cat, despite a clear need for such a treatment.

Although some studies have demonstrated efficacy of other drug classes for DJD-associated pain in dogs (Lascelles *et al.* 2008), evidence indicates that NSAIDs are still currently considered the most effective therapy for pain (Innes *et al.* 2010; Aragon *et al.* 2007; Sanderson *et al.* 2009). However, NSAIDs are not always sufficiently effective in dogs (Lascelles *et al.* 2008) and concerns about side-effects result in a large unmet need in the treatment of canine DJD-associated pain. In cats, there are greater concerns about the use of NSAIDs for long periods of time, especially as the majority of cats presenting with DJD-associated pain have evidence of chronic kidney disease (Marion *et al.* 2013), and NSAIDs may interfere with renal function (Sparkes *et al.* 2010). Indeed, only two placebo-controlled, blinded clinical studies of the efficacy of meloxicam in cats have been published (Gruen *et al.* 2015; Lascelles *et al.* 2007). Because of these concerns, doses lower than the European-approved dose of meloxicam (0.05mg/kg) have been assessed, with evidence from one blinded, placebo-controlled study demonstrating that 0.035mg/kg daily produced measurable improvement over a three-week period of administration (Gruen *et al.* 2015).

An Alternative Approach: Targeting Nerve Growth Factor (NGF)

Nerve growth factor (NGF) has emerged as a potentially useful therapeutic target for pain control. NGF was identified as a protein growth factor critical for the development and maintenance of sensory and sympathetic neurons in the developing nervous system. However, it is now clear that NGF has an important role in pro-nociception (reviewed in: Hefti *et al.* 2006).

NGF binds to the high-affinity NGF-specific receptor TrkA, resulting in autophosphorylation of the TrkA intracellular domain and activation of subsequent downstream signalling cascades (Hefti *et al.* 2006). In nerves, this results in post-translational changes in the transient receptor potential vanilloid receptor 1 (TRPV1) cation channel, lowering its threshold for stimulation. NGF-induced upregulation of other proteins also increases the excitability of the primary afferent fibre (Hefti *et al.* 2006). NGF also activates mast cells, which can further sensitise neurons as a result of the mast cell products released (Kawamoto *et al.* 2002). Given its role in nociception, various methods of preventing activation of TrkA have been explored, including blocking NGF binding to TrkA and preventing activation of TrkA (Eibl *et al.* 2012). Of these approaches, monoclonal antibodies (mAbs) that target and neutralise NGF (blocking binding to receptor) have been developed first (Figure 1).



Inhibition of NGF function via anti-NGF antibodies markedly reduces hyperalgesia and behavioural indicators of pain in various animal models of inflammatory arthritis (Shelton *et al.* 2005; Ghilardi *et al.* 2012). In human clinical studies, several anti-NGF mAbs have been shown to reduce pain and improve function in patients with OA, and these antibodies are currently in late-stage (Phase III) clinical development (Balanescu et al. 2014; Tiseo *et al.* 2014; Sanga et al. 2013; Brown *et al.* 2012; Lande *et al.* 2010).

Development of Anti-NGF Antibodies for Chronic Pain Control in Cats and Dogs

Antibody drugs are large glycoproteins that need to be species-specific, so as to prevent the body developing an immune response to the drug (immunogenicity). Biotechnology company Nexvet has developed an efficient way of creating species-specific antibodies using a process termed PETization (see "About PETization" at Nexvet.com). Briefly, in order to convert anti-NGF antibodies generated in rats (donor mAbs) into cat- and dog-specific antibodies, changes were made to the donor mAb heavy and light chain (NGF-binding) variable domain sequences.
These changes were chosen by alignment of the donor framework sequences with a collection of predicted protein sequences encoded by expressed antibody DNA sequences from the target species (cat or dog), followed by substitution of the most suitable amino acids into the protein sequence. This process ensures retention of the antibody's affinity for NGF and reduces the immunogenic potential of

the donor mAb in the cat or dog. By this process, the rat donor framework sequences are completely "caninised" or "felinised", with minimal changes made from the donor antibody, thus improving the likelihood that converted mAbs retain their affinity and potency. Further engineering of mAb heavy and light chain constant domains enabled the construction of the complete antibodies for dogs and cats, respectively (Gearing *et al*. 2013; 2016).

Anti-NGF Monoclonal Antibody for Chronic Pain in the Dog

A canine-specific mAb against NGF (ranevetmab or NV-01) was generated via PETization and has demonstrated high affinity and potency, no immune cell effector activity, a long half-life and low immunogenic potential (Gearing et al. 2013). An exploratory clinical study provided evidence that NV-01 (0.2 mg/kg intravenously (IV)) alleviated the signs of pain in dogs with OA using a validated pain and mobility assessment questionnaire (the Canine Brief Pain Inventory (Brown *et al.* 2008)) with owners blinded to the time of administration of the antibody (Webster *et al*. 2014). Subsequently, a randomised, double-blinded, placebo-controlled clinical study assessed pain control and effects on mobility of NV-01 in dogs with DJD-associated pain using pain and mobility assessment questionnaires and actimetry (activity monitoring using collar-mounted accelerometers) (Lascelles *et a*l. 2015).

Twenty-six dogs with DJD-associated pain received a single dose of NV-01 (0.2 mg/kg IV) or placebo on day 0 and were then assessed at two and four weeks post-dosing. In addition to objective actimetry measures, owners completed various subjective pain and mobility assessment questionnaires (client-specific outcome measures [CSOM], canine brief pain inventory [CBPI] and Liverpool osteoarthritis in dogs index [LOAD]) on D0, D14 and D28. CBPI scores significantly ($P<0.05$) improved in the NV-01-treated group compared to the placebo-treated group at D14 and D28. The magnitude of the effect was similar to that previously observed with NSAID treatment. CSOM and LOAD scores showed similar improvement in the NV-01-treated group at D14 and D28. No adverse side-effects were noted and neutralising anti-NV-01 antibody responses were not detected. The accelerometer data demonstrated that the average daily activity of animals in the NV-01-treated group increased over the study period compared to placebo, and significant differences were observed during the daytime (9am-5pm). These pilot data supported further clinical assessment of the anti-NGF mAb as an analgesic in dogs suffering from DJD-associated pain.

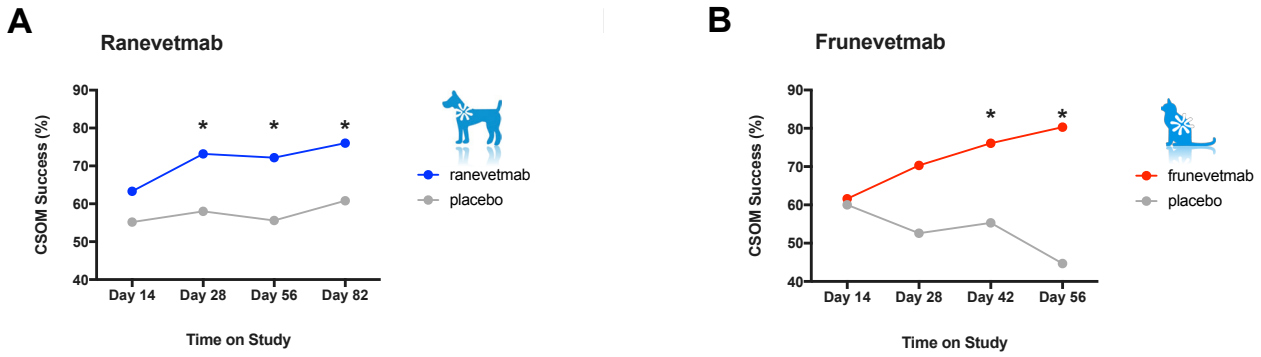
These pilot study results were confirmed in a larger pivotal efficacy and field safety study which enrolled 262 dogs with naturally-occurring osteoarthritis. This randomised, double-blind, placebo-controlled study involved administration of three doses of 0.2 – 0.4 mg/kg of NV-01/ranevetmab delivered at 28-day intervals by subcutaneous injection (SC). Dogs were randomly assigned to receive either NV-01 or placebo at a 2:1 ratio. CSOM and CBPI were the primary assessment tools, conducted at days 0 (pre-first dose), 14, 28, 56 and 84. A statistically significant improvement in pain score was observed in the antibody-treated group compared to the placebo group using pre-determined CSOM improvement success/fail criteria between enrolment and day 28 (this was the primary endpoint of the study as agreed under protocol concurrence with FDA CVM). Statistical significance ($P<0.05$) by this measure was also seen on days 56 and 84. Median change in CSOM scores also showed significant improvement in the antibody-treated dogs for D0 to D28 and D0 to D56 ($p<0-05$). The CSOM global assessment measure, which assesses overall owner experience with the treatment course, achieved statistical significance at the single timepoint at which it was measured, D28. Statistically significant improvements on the assessed level of pain as measured using CBPI treatment success/fail criteria were also seen between enrolment and D14, D28 and D56. No adverse safety signals were associated with anti-NGF mAb administration in this study.

Anti-NGF Monoclonal Antibody for Chronic Pain in the Cat

Following the encouraging outcomes seen in early canine studies of NV-01/ranevetmab, a felinised version of an anti-NGF antibody was designed (termed NV-02/frunevetmab). Similar to the canine antibody, NV-02 has high affinity and potency and was found to be safe with favourable pharmacokinetics in pre-clinical testing in cats. In a placebo-controlled, blinded clinical study, thirty-four client-owned cats with DJD-associated pain and mobility impairment were randomised to a single SC treatment with NV-02 (n=23) or placebo (n=11). Activity was measured objectively using collar-mounted accelerometers, and subjectively by owners completing two clinical pain and mobility questionnaires (CSOM and feline musculoskeletal pain index [FMPI]) on days 0 (at screening), 14 (baseline, NV-02 administration), 35, 56 and 77. NV-02 significantly increased activity overall and at two, three, four, five and six weeks following treatment compared with placebo. CSOM scores were significantly improved three weeks following administration with antibody (Gruen et al. 2016). At D77, 83% of the owners in the NV-02-treated group correctly identified the treatment administered (NV-02 or placebo) compared to 45% of owners in the placebo group. No treatment-related adverse effects were identified. These pilot data demonstrated a positive analgesic effect of six-week duration following anti-NGF antibody administration to the cats.

A larger placebo-controlled, double-blinded pilot field study which enrolled 126 cats with naturally-occurring osteoarthritis was conducted, and further supported the safety and efficacy of NV-02/frunevetmab. This study used CSOM and FMPI as primary assessment tools, at screening, day 0 (first dosing) and days 14, 28, 42 and 56. Two doses of 0.1 – 0.28 mg/kg NV-02/frunevetmab were administered with a 28-day interval between doses. Cats randomly received either NV-02 or placebo at a 2:1 ratio. IV and SC administration were examined: both routes of administration were highly effective and the groups were combined for analysis.

Analysis of the combined NV-02-treatment groups compared to placebo yielded a number of statistically significant ($P<0.05$) improvements, including the assessed level of pain as measured using pre-determined CSOM improvement success/fail criteria between enrolment and both D42 and D56. There were also statistically significant reductions in the assessed level of pain as measured using changes in median total CSOM score between enrolment and day 42 and day 56. A statistically significant difference between NV-02/frunevetmab-treated cats and placebo was seen for the CSOM global assessment on both days it was measured: D28 and D56. Pre-determined FMPI improvement success/fail criteria also showed statistically significant improvement on the assessed level of pain between enrolment and both D42 and D56. Reductions in median FMPI total score showed statistically significant improvement over placebo on the assessed level of pain at D42 and D56. No adverse safety signals associated with NV-02/frunevetmab administration were seen in this study.



Conclusion

These pre-clinical and clinical studies support the hypothesis that species-specific anti-NGF monoclonal antibodies can control pain in dogs and cats, when administered by monthly injection. The high prevalence of conditions that cause chronic pain and disability in these species, combined with a need for differentiated pain management therapeutic options, makes this approach a promising area of development.

Note: The monoclonal antibodies NV-01/ranevetmab and NV-02/frunevetmab remain in clinical development and have not yet been approved for use by a regulatory agency

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