Journal of Veterinary Internal Medicine

Open Access

J Vet Intern Med 2016;30:1129–1137

In Vitro and In Vivo Characterization of a Fully Felinized Therapeutic Anti-Nerve Growth Factor Monoclonal Antibody for the Treatment of Pain in Cats

D.P. Gearing, M. Huebner, E.R. Virtue, K. Knight, P. Hansen, B.D.X. Lascelles, R.P. Gearing, and A.C. Drew

Background: Limited options are available for the treatment of pain in cats. Monoclonal antibodies (mAbs) that neutralize nerve growth factor (NGF) have demonstrated analgesic capacity in rodent models, people with osteoarthritis, and dogs with degenerative joint disease.

Hypothesis/Objectives: This study describes the design and characterization of a fully felinized anti-NGF monoclonal antibody. In vitro potency, pharmacokinetics, and the ability of the antibody to treat pain in a self-resolving, acute inflammation model were investigated in cats.

Animals: Thirty-eight cats at a research colony at Charles River Laboratories, Ireland.

Methods: Felinized anti-NGF mAb, NV-02, was produced using a complementary DNA (cDNA)-based method (PETization). Purified NV-02 was tested for affinity, potency, and immunoreactivity in vitro, then for safety and plasma pharmacokinetic distribution in vivo, and analgesic efficacy in a model of kaolin-induced inflammatory pain.

Results: Anti-NGF mAb, NV-02 neutralized NGF with high affinity and potency and did not bind complement. NV-02-administered SC had a plasma half-life of 7–15 days and was well tolerated at dosages up to 28 mg/kg. A dosage of 2 mg/kg NV-02 SC significantly decreased signs of lameness on day 2 ($P = .0027$), day 3 ($P = .016$), day 4, ($P = .0063$), day 5 ($P = .0085$), day 6 ($P = .0014$), and day 7 ($P = .0034$) after induction of inflammation.

Conclusions and Clinical Importance: The high affinity, long plasma half-life, safety, and analgesic efficacy of felinized anti-NGF mAb (NV-02) support further investigation of the analgesic potential of this antibody in the cat.

Key words: Cat pain; Companion animals; Feline analgesia; PETization; Pharmacokinetics.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used commonly in other mammals for pain relief, but are not widely used for control of pain in cats because of safety concerns.[1] In the United States, no NSAIDs are approved for chronic use in cats, and in the European Union (EU), only 1 NSAID (meloxicam) is approved for the treatment of chronic pain. However, in the United States, meloxicam has a boxed warning, cautioning against repeated dosing.

Anti-nerve growth factor (NGF) monoclonal antibodies (mAbs) have been shown to have analgesic effects in rodent models of pain,[2] in several human clinical trials[3–5] and, more recently, in proof-of-concept clinical studies in osteoarthritic dogs.[6–8] Long-acting pain relief (>4 weeks) and good tolerability was observed in the dog studies after a single injection.

Although no published data are available linking NGF and pain in cats, the amino acid sequences of human and feline NGF are highly conserved (Fig 1A). Given the long half-life of mAbs in mammals in general, the favorable safety of humanized antibodies as a class, and the potential for an equivalent role of NGF in mediating pain in cats, as in other mammals, we reasoned that a felinized mAb (ie, designed to be recognized as self by the feline immune system) that neutralized feline NGF might have potential as a long-acting analgesic in cats and potentially would have an

Abbreviations:

BBB	blood-brain barrier
BSA	bovine serum albumin
CDR	complementarity-determining region
CHO	Chinese hamster ovary
DJD	degenerative joint disease
ELISA	enzyme-linked immuno-sorbent assay
IV	intravenous
mAb	monoclonal antibody
NGF	nerve growth factor
NSAID	nonsteroidal anti-inflammatory drug
OA	osteoarthritis
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
PK	pharmacokinetic
SC	subcutaneous
SDS	sodium dodecyl sulfate
SPR	surface plasmon resonance
TMB	tetramethylbenzidine

From Nexvet Australia Pty. Ltd., Melbourne, VIC Australia (DP Gearing, Virtue, Knight, Hansen, Drew); ClinData Services Inc., Fort Collins, CO (Huebner); the Comparative Pain Research Program, College of Veterinary Medicine, North Carolina State University, (Lascelles); the Comparative Medicine Institute, North Carolina State University, Raleigh, (Lascelles); the Center for Pain Research and Innovation, UNC Chapel Hill, Chapel Hill, NC (Lascelles); and Ridge Biotechnology, Seattle, WA (RP Gearing).

Corresponding author: D.P. Gearing, Nexvet Australia Pty Ltd, 31 Queen Street, Melbourne, VIC 3000, Australia; e-mail: dave.gearing@nexvet.com

Submitted January 31, 2016; Revised March 31, 2016; Accepted May 5, 2016.

DOI: 10.1111/jvim.13985

improved safety profile compared to existing therapies. In this study, we describe the properties of such a felinized anti-NGF mAb in vitro and in vivo, including its activity in alleviating signs of pain in a short-term, self-resolving model of inflammation in cats.

Materials and Methods

Conversion of an Anti-NGF Antibody for Use in the Cat

Monoclonal antibodies generated by immunization of rodents are immunogenic if injected into other mammals. For use in humans, rodent mAbs are modified for injection by a process termed "humanizing" or "humanization". By analogy, a rodent mAb converted for treatment in the cat would be termed "felinized". To decrease the immunogenic potential of rat anti-mouse NGF mAb αD11[9] in the cat, while retaining its high affinity for NGF, amino acid substitutions were made to the heavy and light chain variable domain framework sequences by alignment with a collection of predicted protein sequences encoded by expressed feline immunoglobulin (IgG) complementary deoxyribonucleic acid (cDNA) sequences. This approach (that we refer to as PETization) previously was used to generate a fully caninized anti-NGF mAb that has a promising efficacy and safety profile in dogs.[6–8] Where an amino acid in the αD11 sequence corresponded to an amino acid in the collection no change was made. Where it differed, the most similar amino acid (eg, by charge or size) at that position in the collection was substituted. If no similar amino acid was

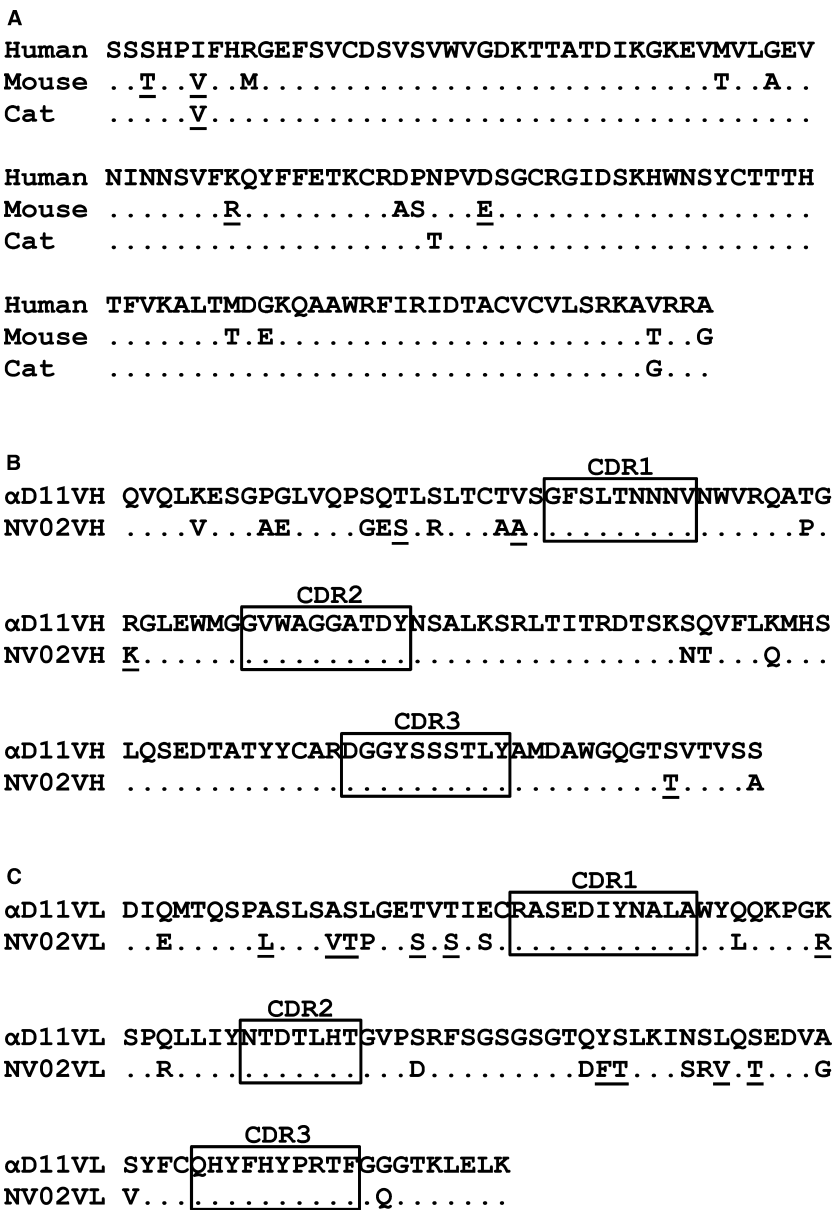

Fig 1. NGF and anti-NGF antibody sequences. (**A**) Alignment of the mature peptide sequence of NGF from human, mouse, and cat. Identical amino acids are indicated by dots and similar amino acids are underlined. (**B**) Variable heavy and (**C**) variable light chain sequences of the anti-NGF antibody αD11 aligned to the felinized antibody NV-02. Identical amino acids are indicated by dots and similar amino acids are underlined. Complementarity-determining regions (CDR) are boxed.

available, the most abundant feline amino acid residue at that position was chosen. The changes made are illustrated in Fig 1B and C. Sixteen substitutions were made to the heavy chain variable domain, of which 4 were conservative (ie, related by charge, size, or hydrophobicity) and 22 substitutions were made to the light chain variable domain, of which 10 were conservative. By this process, the αD11 framework amino acid sequences were completely felinized, with minimal changes made from the donor αD11 antibody. The felinized αD11 heavy chain variable domain sequence was combined with an immunoglobulin heavy chain signal sequence and the constant domain sequence of feline IgG heavy chain isotype IgG1 to form the feN-HC1 sequence. The felinized NV-02 light chain variable domain sequence was combined with a light chain signal sequence and the constant domain sequence of the feline kappa light chain to form the feN-kLC sequence. The resulting amino acid sequences were converted to Chinese Hamster Ovary (CHO) cell codon-optimized nucleotide sequences and cloned separately into pcDNA3.1+ for expression in CHO cells.[a] For small-scale work, antibodies (NV-02, NV-01 and caN-HCB-kLC1) were transiently expressed in CHO cells[a] and purified by protein A chromatography from transfected CHO cell supernatant. For larger scale in vivo experiments requiring more material, NV-02 antibody was stably expressed in CHO cells.[b] Stable CHO cell lines with high productivity were prepared with cDNA encoding NV-02 heavy and light chains, and were cultured in a fed batch system, before harvesting of supernatant containing NV-02. The protein was purified by MabSelectSuRe[c] and Sartobind Q[d] chromatography, then concentrated and formulated into phosphate-buffered saline (PBS) pH 7.2. Endotoxin concentrations were determined using an Endosafe®-PTS™ kit.

In Vitro Characterization of the Felinized Anti-NGF Antibody

Complement C1q binding was assayed as previously described.[10] Plates were coated with 2.5 μg/mL mouse NGF and blocked with 5% bovine serum albumin (BSA)/phosphate-buffered saline (PBS). Coated wells were incubated for 1 hour at room temperature with NV-02 antibody, or as a positive control a caninized anti-NGF mAb with a IgG-B isotype (complement-binding) heavy chain,[6] diluted in PBS/1% BSA. Antibody concentrations ranged from 10 μg/mL to 1.0 μg/mL. The plates were washed and incubated for 1 hour at room temperature with human serum or heat-inactivated human serum diluted 1/100 in veronal-buffered saline containing 0.05% Tween-20, 0.1% gelatine, and 0.5% BSA. After washing, plates were incubated with a 1/800 dilution of sheep anti-human C1q-HRP[e] in PBS/1% BSA. After washing, plates were developed by the addition of tetramethylbenzidine (TMB) substrate.[f] Development was stopped by the addition of 2M H_2SO_4 and absorbance read at 450 nm. The absorbance at 450 nm obtained using heat-inactivated serum was used as background and subtracted from the absorbance at 450 nm obtained using untreated serum.

The binding affinity of NV-02 to mouse NGF was analyzed by surface plasmon resonance assay (SPR) using a ProteOn XPR36 SPRi biosensor equipped with a GLM chip[f]. The chip was conditioned with 0.5% sodium dodecyl sulfate (SDS), 50 mM NaOH, and 100 mM HCl. After conditioning, the lanes were activated using equal parts of 1-ethyl-3-(-3-demethylaminopropyl) carbodiimide and N-hydroxysulfosuccinimide amine coupling reagents. The NGF protein was immobilized to the chip at a concentration of 50 μg/mL in sodium acetate buffer (pH 4.5). After immobilization all 3 channels were deactivated using ethanolamine. The NV-02 was passed across the surface at 500 nM, 250 nM, 125 nM, 62.5 nM, and 31.25 nM. The binding was displayed as a spectrogram. Controls were subtracted to give specific binding. A Langmuir curve fit model then was used to determine the specific affinity. Inhibition of NGF binding by NV-02 was assessed using TF-1 cells as previously described.[6] TF-1 cells were starved for 24 hours, then cultured in 96-well plates in media supplemented with 1 ng/mL mouse NGF and increasing concentrations of NV-02 or caninized anti-NGF mAb NV-01. The plates were incubated for 48 hour at 37°C/5% CO_2 before measuring proliferation using a CellTiter 96 Aqueous One Solution Cell Proliferation Assay.[g] The assay was performed in triplicate. A mouse IgG2a mAb[h] was used as a negative control.

Pharmacokinetics and Safety of NV-02 In Vivo

Pharmacokinetic (PK) studies were conducted in a research colony of cats at Charles River Laboratories (CRL), Ireland, after institutional ethics review panel approval (code CRL/001-15/001). Purified NV-02 was injected SC, at 2.0 mg/kg, 5.6 mg/kg, 16.8 mg/kg, or 28 mg/kg into 8 cats (n = 2 animals/group) and plasma samples were taken over the next 42 days. The concentration of NV-02 in the plasma samples was determined using a quantitative NGF-binding ELISA. Immunoassay plates (F96 Maxisorp[i]) were coated with 100 μL of 0.1 μg/mL mouse NGF in PBS and blocked with PBS/0.05% Tween 20/1% BSA. Assay diluent was prepared by diluting pooled normal cat plasma in PBS/0.05% Tween 20/1% BSA. Standards were prepared by diluting NV-02 in assay diluent to 40, 30, 20, 10, 5, 2, and 1 ng/mL. Quality control (QC) samples were prepared from independent stock solutions of NV-02 by diluting in assay diluent to final concentrations of 30, 15, and 3 ng/mL. Two individual dilutions were prepared for each QC level. Samples were diluted to the required dilution in PBS/0.05% Tween 20/1% BSA.

The NGF-coated wells were incubated for 1 hour at room temperature with standard curve, QC and plasma samples. After washing with PBS/0.05% Tween 20, the plates were incubated with a 1/10,000 dilution of goat anti-feline IgG (heavy and light chain specific)-horseradish peroxidase conjugate[j] in PBS/0.05% Tween 20/1% BSA for 1 hour. Plates were washed and developed by the addition of TMB substrate[i]. Development was stopped by the addition of 2M H_2SO_4. The assay was analyzed using a 4 parameter fit. An assay was considered to pass if 4 of 6 QC samples were within 20% of the expected concentration, including 1 of each QC concentration. Plasma half-life was determined using the program PK Solver (11).

Safety was assessed during the course of the study by the CRL veterinarians, observing behavior (daily) and measuring weight (daily), clinical chemistry variables (days 14 and 42: glucose, creatine phosphokinase, sodium, chloride, potassium, phosphate, cholesterol, creatinine, total protein, albumin, globulin, calcium), and hematology assessments (days 0, 14, and 42: red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cells, lymphocytes, monocytes, neutrophils, eosinophils, basophils, large unstained cells).

Model of Inflammatory Pain

A validated, reversible, and ethical model of inflammatory pain in the cat induced by injection of kaolin into the footpad[11] was used, after institutional ethics review and approval at CRL, Ireland (CRL/001-14/004). After kaolin injection into the footpad, a pain-induced lameness develops in that leg within 24 hour that progressively recovers over a period of 7–14 days. Cats were then returned to the colony. After review and approval by the CRL institutional ethics committee, the kaolin model was used to assess the analgesic potential of a single 2 mg/kg dose of NV-02-

delivered SC. Vehicle (PBS) was administered SC as a negative control. To maintain blinding, the administration of NV-02 or vehicle was performed by investigators different from those who assessed lameness. The investigators involved in the lameness assessments were masked to the treatments administered to decrease bias. The investigators involved in the administration of NV-02 or vehicle control were not masked.

Thirty cats were used and allocated to 2 treatment groups (n = 15 per group). Animals assigned to Group 1 served as a negative control group and were treated with PBS administered by SC injection in the neck region. Animals assigned to Group 2 were treated with NV-02 administered by SC injection at a dosage of 2.0 mg/kg. To facilitate peak plasma concentrations of NV-02 (based on PK data, Fig 4) and optimal distribution to the footpad, NV-02 was given 4 days before a kaolin injection. On the day of kaolin administration to the cats, general anesthesia was induced using ketamine (approximately 7.5 mg/kg [0.075 mL/kg]) and medetomidine (approximately 0.08 mg/kg [0.08 mL/kg]) by IM injection. After kaolin injection, any cat that had not fully recovered after 45 minutes of sedation was given atipamezole hydrochloride (0.2 mg/kg [0.04 mL/kg]) IM to reverse the sedation. One cat did not recover from the sedation and this event was attributed to anesthetic death. The cats underwent experimental induction of paw inflammation using 5.4 mL of 250 mg/mL kaolin^{k} /PBS solution injected SC in portions at 6 sites in the right hind paw. The degree of lameness induced by kaolin was scored according to 4 levels using a discontinuous scoring system,[6,11] where a score of 0 = full weight bearing; 1 = slightly lame (not fully weight bearing but walking well); 2 = moderately lame (slightly weight bearing and not walking well); and 3 = severely lame (not weight bearing). All animals used in the study became lame 24 hour after kaolin administration. Lameness assessments were performed at the following times: before kaolin administration, pretreatment on study day 0; and at the following times after dosing: 6h, +1d, +2d, +3d, +4d, +5d, +6d, and +7d. The lameness scores were unblinded and scores from NV-02-treated cats were compared to the placebo control-treated cats as described previously.[6] Circumference measurements of the kaolin-injected paw and rectal temperature measurement were taken daily and averaged across each group.

Statistical Methods (in vivo kaolin experiment)

Descriptive statistics (number of subjects, mean, standard deviation, standard error of the mean, minimum, median, and maximum values) were determined. Repeated measures analysis of variance (ANOVA) methods were employed. The model included study day as a fixed effect and animal identification as a random effect. Assumptions of normality of residuals were investigated using the Shapiro-Wilk test. If the P value was $< .05$ then it was determined that the distribution cannot be approximated by a normal curve. The values then were ranked in ascending order with tied values given a mean rank before running statistical models. The covariance structure that provided the smallest Akaike's information criterion (AIC) was selected. Pairwise comparison of the active dose to placebo was generated from the repeated measures ANOVA model.

Results

Characterization of Felinized Anti-NGF mAb In Vitro

The felinized anti-NGF mAb NV-02 heavy and light chain cDNA sequences were subcloned into a mammalian expression vector and transfected into CHO cells. Purified NV-02 mAb was isolated from transfected CHO cell supernatants (previously cultured in animal component-free chemically defined media) by Protein A affinity chromatography, ion-exchange chromatography, and sterile filtration. This procedure resulted in highly purified preparations of NV-02 (99.3% monomer by size-exclusion high-performance liquid chromatography with low endotoxin concentrations (<0.1 EU/mg).

Purified NV-02 mAb was assayed by size-exclusion fast protein liquid chromatography (FPLC) and shown to consist predominantly of a monomeric species with an apparent molecular weight of approximately 150 kDa (Fig 2A), which was confirmed by non-reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Analysis by reducing SDS-PAGE identified heavy and light chains of approximately 50 kDa and 25 kDa, respectively, as expected (Fig 2B). Unexpected heterogeneity of the light chains was assessed by N-glycanase treatment of NV-02 that resulted in a

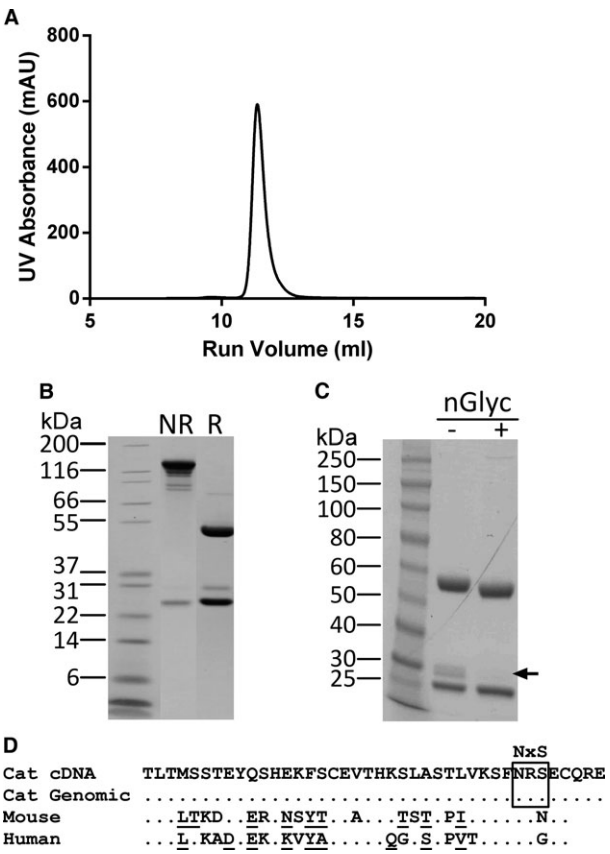

Fig 2. Characterization of purified felinized anti-NGF antibody NV-02. (**A**) Size-exclusion chromatography of purified NV-02. (**B**) SDS-PAGE of nonreduced (NR) and reduced (R) NV-02. Corresponding molecular weight standards are identified (numbers in kDa). (**C**) Glycosylation analysis of NV-02. NV-02 (nGlyc −) was treated with N-Glycanase (nGlyc +) and analyzed by reducing SDS-PAGE. The arrow indicates the position of glycosylated light chain variants that are absent after treatment. (**D**) Comparison of the translated cDNA and genomic DNA-derived protein sequences of the C-terminal region of the cat kappa light chain showing the presence of a glycosylation site (boxed, NxS) not found in the kappa light chain of mouse or human IgG.

decrease in the apparent molecular weight of both the heavy and light chains (Fig 2C). Analysis of genomic and expressed cDNA feline kappa light chain sequences identified an N-linked glycosylation site close to the C-terminus (Fig 2D) that likely explains the heterogeneity, with some light chains more modified than others. Analysis by mass spectrometry confirmed the presence of a heterogeneous population of glycans on the light chains seen with SDS-PAGE (Fig 2C; not shown).

Purified NV-02 mAb was tested for its ability to neutralize NGF in vitro using an NGF-dependent proliferation assay of TF-1 cells, as previously described.[6] The NV-02 had equivalent potency in this assay as caninized anti-NGF mAb NV-01 (Fig 3A). Surface plasmon resonance (SPR) assays (Fig 3B) indicated high-affinity binding of NV-02 to NGF (K_D = 20 pM), equivalent to NV-01,[6] with no appreciable dissociation of ligand after switching the flow to buffer, indicating that the high affinity of binding was caused by a very slow off rate. Using an ELISA for detection of C1q, the first component of the complement cascade, NGF-captured NV-02, was shown to bind little or no C1q (Fig 3C) by comparison with a C1q-binding control antibody (caN-HCB-kLC1).[6] This result suggests that NV-02 will not initiate complement-mediated immune damage, an important safety consideration before its use in vivo.

Pharmacokinetics and Safety of NV-02 In Vivo

The concentration-time plots of NV-02 in the feline plasma samples are depicted in Fig 4. The PK profiles are typical of IgG injected to other mammals, with an absorption phase from the site of injection to plasma followed by a decay phase corresponding to elimination from the plasma. After absorption from the injection site, peak plasma concentrations (Cmax) were achieved at approximately 3 days (Tmax; range, 1.9–4.3 days). The averaged elimination phase half-life was calculated to be 9 days (range, 7–15 days).

There were no changes in body weights, clinical chemistry measurements, and hematology assessments in any of the cats. No adverse reactions or behavioral changes were noted by the veterinarians.

Effect of NV-02 administration in a Kaolin-induced Model of Inflammatory Lameness

Lameness scores were ranked before generating the repeated measures ANOVA because it was determined that the distribution could not be approximated by a normal curve. The covariance structure that provided the smallest AIC was heterogeneous autoregressive. Pairwise comparisons to placebo yielded statistically significant lower lameness scores in the NV-02 group at day 2 (P = .0027), day 3 (P = .0166), day 4 (P = .0063), day 5 (P = .0085), day 6 (P = .0014), and day 7 (P = .0034). Mean lameness scores are presented in Fig 5. No difference was observed in mean rectal temperature or mean paw circumference measurements between placebo- and NV-02-treated cats on any treatment day (data not shown).

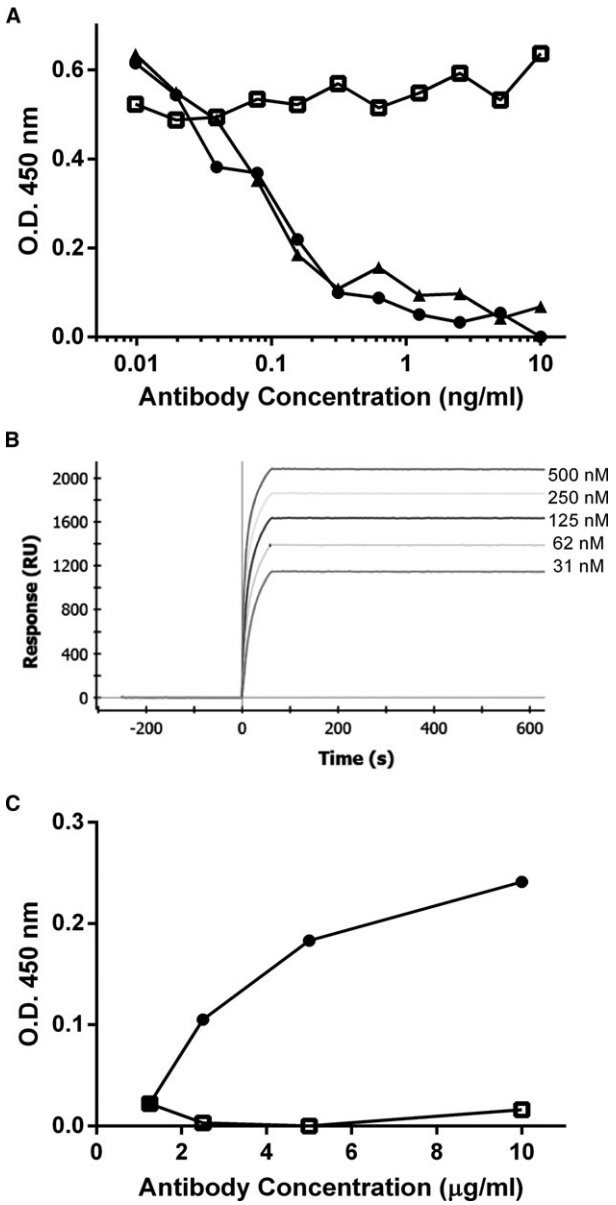

Fig 3. Functional characterization of the felinized anti-NGF antibody NV-02. (**A**) Inhibition of NGF induced proliferation of TF-1 cells in vitro. 1 ng/mL NGF was incubated with NV-02 (●), a caninized anti-NGF mAb (NV-01, ▲) or an irrelevant IgG control antibody (□) at concentrations ranging from 10 to 0.01 ng/mL. (**B**) Binding of NV-02 to immobilized NGF assessed by surface plasmon resonance (SPR). Various concentrations of NV-02 (500 nM, 250 nM, 125 nM, 62.5 nM, and 31.25 nM) were passed over NGF for 60 second then the flow switched to buffer. Relative binding is plotted as response units (RU) over time. (**C**) Complement C1q binding ELISA. NV-02 (□) or a control non-C1q binding caninized anti-NGF mAb, caN-HCB2-kLC1 (●) were bound to immobilized NGF and incubated with serum. C1q binding was detected by polyclonal anti-C1q-HRP.

Discussion

Our study represents the initial characterization of a therapeutic antibody with the potential to treat acute and possibly chronic pain in cats. No pharmaceutical or

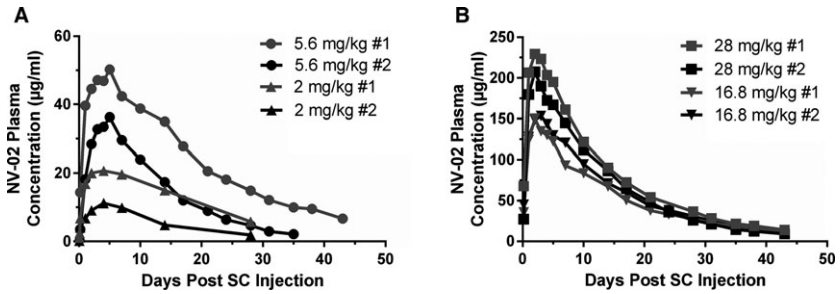

Fig 4. Pharmacokinetic profile of NV-02 in cat plasma following subcutaneous administration at various dose levels. A single dose of NV-02 at 2 mg/kg and 5.6 mg/kg (**A**) or 16.8 mg/kg and 28 mg/kg (**B**) was administered to two cats per dose level and the plasma concentration of NV-02 was assayed at the times indicated by a quantitative NGF-binding ELISA.

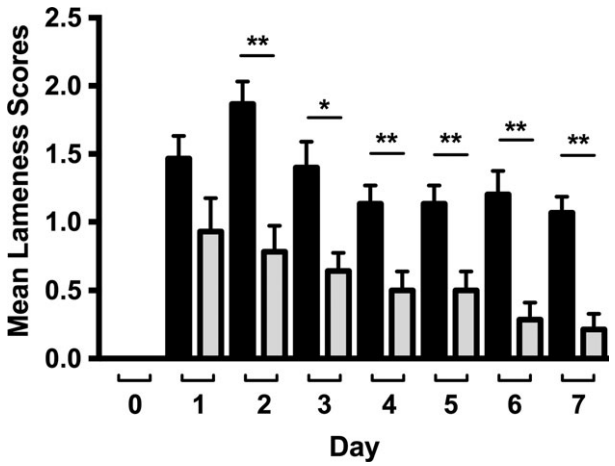

Fig 5. Lameness assessment scores following subcutaneous injection of NV-02 to cats. Cats were injected with vehicle (black bars; N = 15) or 2 mg/kg NV-02 (grey bars; N = 14) on Day-4. Kaolin was administered on Day 0 and lameness was assessed over the following 7 days using a visual analog scoring system. Average lameness scores are presented for each group with error bars representing SEM. Significant *P* values generated by repeated measures ANOVA are denoted $*P < .05$ and $**P < .01$ and indicated above each pair.

biopharmaceutical products are approved by the United States Food and Drug Administration (US-FDA) for long-term control of chronic pain in cats, unlike in dogs, where several NSAIDs are FDA-approved for this indication. This situation exists although chronic pain associated with degenerative joint disease appears to be very prevalent in cats.[13,14] Two NSAIDs, meloxicam and robenacoxib, are FDA-approved for the treatment of postoperative pain in cats, but meloxicam is restricted to a single administration and robenacoxib to a maximum of 3 days. In Europe, meloxicam is approved for unlimited daily use in the cat for the control of musculoskeletal pain, yet the same drug (meloxicam) carries a boxed warning in the United States, which reads, "Repeated use of meloxicam in cats has been associated with acute renal failure and death. Do not administer additional injectable or oral meloxicam to cats". The controversy over repeated administration of meloxicam, or indeed any NSAID, aside, clearly the need for new analgesic medications for the control of chronic pain in cats is high.

Nerve growth factor is a highly conserved dimeric peptide hormone produced by proteolytic cleavage of a pre-pro-peptide precursor. During development, NGF is a neurotrophic factor essential for the survival of sensory and sympathetic neurons.[15,16] In the adult, NGF is expressed at sites of injury and inflammation and is a major factor promoting pain and hyperalgesia.[4,5] Nerve growth factor acts on 2 receptors, the high-affinity trkA receptor and low-affinity p75 receptor. The modulating effect of NGF on nociceptive neurons is mediated by the trkA receptor, resulting in increased immediate and long-term excitability[3] by modulation of ion channels such as the transient receptor potential vanilloid receptor (TRPV1).[3] Nerve growth factor also causes sprouting of nerve endings into the site of inflammation[2] and has been detected in neuromas.[17] Administration of NGF can potentiate pain, and mutations in the trkA receptor are associated with diminished pain responses.[4] Furthermore, antibodies that neutralize NGF are highly effective analgesics in animal models of inflammatory pain, arthritis, cancer pain, and bone fracture.[2,3]

This encouraging biological activity has resulted in several NGF antagonists being developed for the treatment of pain in humans.[3] The clinical efficacy of anti-human NGF mAbs has been demonstrated in several pain states (eg, OA, lower back pain, cystitis) in clinical trials, including large Phase 3 studies.[3–5,18,19] The NGF inhibitory antibodies were generally very well tolerated (consistent with a benign profile in 6-month primate studies[20]) and mild to moderate, transient peripheral sensation changes were the only neurological consequences.[3–5] In 2010, the FDA instructed several companies to put their clinical programs on hold (except those for cancer pain) after observations of worsening of clinical signs in a small proportion of patients with OA, requiring accelerated joint replacement.[21] The cause of this worsening, termed "rapidly progressing OA", was debated, although in some patients, the accelerated OA appeared to be associated with concomitant NSAID use. Nonetheless, the FDA expert review panel overseeing these studies recommended that the clinical halt be lifted in 2012.[22]

The first veterinary application of an anti-NGF mAb recently was described in the form of a fully caninized anti-NGF mAb.[6] This high-affinity mAb (NV-01) also

was derived from αD11[9] and is a potent inhibitor of NGF in vitro. In dogs, the mAb had a long serum half-life, did not generate neutralizing anti-drug antibodies and was effective in decreasing signs of lameness caused by inflammation. In a pilot clinical study of 9 dogs, a single dose of NV-01 mAb was well tolerated and significantly decreased clinical signs of pain and improved mobility in OA dogs, as assessed by owners using the Canine Brief Pain Inventory.[7] The magnitude of improvement in clinical signs was similar to that seen previously with NSAIDs and the duration of effect was at least 4 weeks. More recently, in a blinded, placebo-controlled study of 26 dogs with degenerative joint disease (including OA), a single dose of NV-01 similarly was well tolerated and effective at decreasing pain and improving mobility.[8] The NV-01-treated dogs showed improvement in 3 independent clinical scoring methods, again in magnitude similar to that observed with NSAIDs and over a period of 4 weeks. Furthermore, the NV-01-treated dogs were more active during the daytime, as assessed by collar-mounted accelerometers.

Given its role in mediating inflammatory pain in rodents, dogs, and humans, we reasoned that NGF would be a useful therapeutic target in cats with painful conditions. Feline NGF and its receptor are closely homologous to those of other species. For mature (beta) NGF, there is 100% identity between dog and cat, 91% identity between human and cat, 85% identity between rat and cat, and 82% identity between mouse and cat. Nerve growth factor and its receptor trkA are expressed in similar tissues in cats and humans, appear to be under similar control mechanisms, and have similar functions.[23,24] As with other mammals, the feline immune system shares major immunoglobulin types, including IgG, IgG-Fc receptors including the high-affinity FcR, and the neonatal FcRn, which potentiates IgG half-life in vivo.[25–27]

Felinized anti-NGF mAb NV-02, like the caninized mAb NV-01 previously described, retains the high affinity of its parent mAb αD11 (Fig 3B) and has similar ability to neutralize NGF in vitro (Fig 3A), suggesting that NV-02 retains the structural integrity of the parent mAb. Because the concentration of antibody required to neutralize a given amount of NGF is decreased with increased affinity and potency, the results with NV-02 are predictive of low dosage for in vivo efficacy. Furthermore, NV-02 does not recruit complement (Fig 3C), which is important for its safe use in vivo.

We observed no safety signals in cats injected with a single dose of NV-02 by the SC route at dosages up to 28 mg/kg. Although the safety studies of this small cohort were limited to observations of behavior, weight change, blood chemistry, and hematology, we conclude that NV-02 lacks overt toxicity. Further research will be necessary to confirm this safety profile. Delivery of anti-NGF mAbs SC limits their exposure to the periphery, because mAbs do not cross the blood-brain barrier (BBB). Consequently, action on the central nervous system is avoided. Anti-NGF mAbs from the maternal circulation (via the placenta and developing BBB) cause fetal abnormalities in rodents,[28,29] and in pregnant non-human primates, caused increased stillbirth, postbirth infant mortality and morbidity, decreased infant growth, sensory and sympathetic nervous system changes, and decreased infant primary antibody responses.[30] The use of anti-NGF mAbs should be avoided in pregnant or lactating animals. By contrast, in the adult, very high doses of anti-NGF mAb delivered IV or SC were well tolerated and extensive analysis identified no effects on adult peripheral nerves.[31]

When administered to cats SC, 4 days before kaolin, NV-02 decreased the severity of lameness and maintained protection from lameness over the following week. As previously observed for anti-NGF mAbs in other species,[2] NV-02 did not decrease inflammatory pyrexia or swelling of the paw compared with placebo-treated cats. A similar duration of effect was observed previously with a caninized anti-NGF mAb in the kaolin model in dogs, and longer effects were observed in dogs with degenerative joint disease (DJD). Studies of cats with DJD will be necessary to determine whether NV-02 is effective in providing analgesia in this setting, as well as whether a duration of effect >7 days can be achieved after a single injection.

Monoclonal antibodies have become an important part of the therapeutic treatment options for several diseases of humans including inflammation, autoimmunity, cancer, allergy, and blindness because of their combination of efficacy, duration of effect, and safety. The PETization approach, described here with the anti-NGF mAb for cats (NV-02) and previously with dogs (NV-01) produces mAbs with similar properties to humanized mAbs and potentially will allow the use of mAbs for the treatment of many diseases in veterinary patients.

Conclusions

Our data support the hypothesis that neutralization of NGF in cats with the mAb NV-02 may have the potential to provide appropriately safe analgesia in the cat, potentially for prolonged durations. These conclusions are based on our findings of high affinity for and potent inhibition of NGF in vitro, no evidence of binding complement component C1q, a long plasma half-life, and a lack of observed toxicity in vivo.

Further work will be necessary to assess: (1) the value of this mAb in treating chronic pain states in the cat, such as the pain associated with degenerative joint disease; (2) the optimal route of delivery, dose, and dosing strategy for best efficacy; and (3) its safety in larger cohorts of normal or diseased cats.

The mAb NV-02 has the potential to provide an advance in the treatment of many cats with joint pain associated with OA and other forms of DJD. While this paper was under review, a paper describing the safety and efficacy of NV-02 mAb in cats with degenerative joint disease has been published in JVIM (Gruen et al. 2016). USAN have adopted the non-proprietary name frunevetmab for anti-NGF mAb NV-02.

Footnotes

[a] Geneart AG, Life Technologies, Regensburg, Germany
[b] Lonza Biologics plc, Cambridge, UK
[c] GE Healthcare, UK
[d] Sartorius, Australia
[e] AbD Serotec, UK
[f] BioRad, Hercules
[g] Promega, Madison
[h] eBM2a, eBioscience, San Diego
[i] ThermoFisher Scientific
[j] Jackson Immunoresearch Laboratories
[k] Sigma-Aldrich

Acknowledgments

The authors thank the following individuals for their contributions to this study: Paul Hertzog, Kevin Johnson, Angel Lopez, and colleagues at Nexvet for technical advice and support; John Cox, Jane Eagleson, Colin Giles, Andy Gearing, and Samantha Busfield for critical review of the manuscript; Michael Spring and colleagues (Life Technologies, Germany) for gene synthesis services and small-scale protein expression; Antony Munn and colleagues (Lonza, UK) for protein expression and purification; Edouard Nice and Daniel Layton (MATF, Monash University) for binding affinity studies; and colleagues at Charles River Laboratories (Ballina, Ireland) for performing the animal studies and kaolin model.

Conflict of Interest Declaration: All authors except MH and BDXL are employees, stockholders, or both of Nexvet Australia Pty Ltd. MH, BDXL, and RPG are paid consultants of Nexvet Australia Pty Ltd.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References

1. Sparkes AH, Heiene R, Lascelles BD, et al. ISFM and AAFP consensus guidelines: Long-term use of NSAIDs in cats. J Feline Med Surg 2010;12:521–538.

2. Ghilardi JR, Freeman KT, Jimenez-Andrade JM, et al. Neuroplasticity of sensory and sympathetic nerve fibers in a mouse model of a painful arthritic joint. Arth Rheum 2012;64:2223–2232.

3. Cattaneo A. Tanezumab, a recombinant humanized mAb against nerve growth factor for the treatment of acute and chronic pain. Curr Opin Mol Ther 2010;12:94–106.

4. Hefti FF, Rosenthal A, Walicke PA, et al. Novel class of pain drugs based on antagonism of NGF. Trends Pharmacol Sci 2006;27:85–91.

5. Mantyh PW, Koltzenburg M, Mendell LM, et al. Antagonism of nerve growth factor-TrkA signaling and the relief of pain. Anesthesiology 2011;115:189–204.

6. Gearing DP, Virtue ER, Gearing RP, Drew AC. A fully caninised anti-NGF monoclonal antibody for pain relief in dogs. BMC Vet Res 2013;9:226.

7. Webster RP, Anderson GI, Gearing DP. Canine Brief Pain Inventory scores for dogs with osteoarthritis before and after administration of a monoclonal antibody against nerve growth factor. Am J Vet Res 2014;75:532–535.

8. Lascelles BD, Knazovicky D, Case B, et al. A canine-specific anti-nerve growth factor antibody alleviates pain and improves mobility and function in dogs with degenerative joint disease-associated pain. BMC Vet Res 2015;11:101.

9. Ruberti F, Bradbury A, Cattaneo A. Cloning and expression of an anti-nerve growth factor (NGF) antibody for studies using the neuroantibody approach. Cell Mol Neurobiol 1993;13:559–568.

10. Lewis MJ, Wagner B, Woof JM. The different effector function capabilities of the seven equine IgG subclasses have implications for vaccine strategies. Mol Immunol 2008;45:818–827.

11. Giraudel JM, Diquelou A, Laroute V, et al. Pharmacokinetic/pharmacodynamic modelling of NSAIDs in a model of reversible inflammation in the cat. Br J Pharmacol 2005;146:642–653.

12. Zhang Y, Huo M, Zhou J, Xie S. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. Comput Methods Programs Biomed 2010;99:306–314.

13. Lascelles BD, Henry JB, Brown J, et al. Cross-sectional study of the prevalence of radiographic degenerative joint disease in domesticated cats. Vet Surg: VS 2010;39:535–544.

14. Slingerland LI, Hazewinkel HA, Meij BP, et al. Cross Sectional study of the prevelance and clinical features of osteoarthritis in 100 cats. Vet J 2011;187:304–309.

15. Levi-Montalcini R. The nerve growth factor 35 years later. Science 1987;237:1154–1162.

16. Levi-Montalcini R, Skaper SD, Dal Toso R, et al. Nerve growth factor: From neurotrophin to neurokine. Trends Neurosci 1996;19:514–520.

17. Harpf C, Dabernig J, Humpel C. Receptors for NGF and GDNF are highly expressed in human peripheral nerve neuroma. Muscle Nerve 2002;25:612–615.

18. Lane NE, Schnitzer TJ, Birbara CA, et al. Tanezumab for the treatment of pain from osteoarthritis of the knee. New England J Med 2010;363:1521–1531.

19. Katz N, Borenstein DG, Birbara C, et al. Efficacy and safety of tanezumab in the treatment of chronic low back pain. Pain 2011;152:2248–2258.

20. Zorbas M, Hurst S, Shelton D, et al. A multiple-dose toxicity study of tanezumab in cynomolgus monkeys. Regul Toxicol Pharmacol: RTP 2011;59:334–342.

21. Garber K. Fate of novel painkiller mAbs hangs in balance. Nat Biotechnol 2011;29:173–174.

22. United States Food and Drug Administration. Summary minutes of the arthritis advisory committee meeting March 12, 2012. Available from http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/ArthritisAdvisoryCommittee/UCM307879.pdf. Accessed November 2013.

23. Fried K, Risling M, Arvidsson U, Paulie S. Nerve growth factor receptor-like immunoreactivity in nerve fibers in the spinal and medullary dorsal horn of the adult monkey and cat: Correlation with calcitonin gene-related peptide-like immunoreactivity. Brain Res 1990;536:321–326.

24. Fried K, Risling M. Nerve growth factor receptor-like immunoreactivity in primary and permanent canine tooth pulps of the cat. Cell Tissue Res 1991;264:321–328.

25. Hinton PR, Johlfs MG, Xiong JM, et al. Engineered human IgG antibodies with longer serum half-lives in primates. J Biol Chem 2004;279:6213–6216.

26. Mould DR, Sweeney KR. The pharmacokinetics and pharmacodynamics of monoclonal antibodies–mechanistic modeling applied to drug development. Curr Opin Drug Discov Devel 2007;10:84–96.

27. Strietzel CJ, Bergeron LM, Oliphant T, et al. In vitro functional characterization of feline IgGs. Vet Immunol Immunopathol 2014;158:214–223.

28. Aloe L, Cozzari C, Calissano P, Levi-Montalcini R. Somatic and behavioral postnatal effects of fetal injections of nerve growth factor antibodies in the rat. Nature 1981;291:413–415.

29. Pearson J, Johnson EM, Brandeis L. Effects of antibodies to nerve growth factor on intrauterine development of derivatives of cranial neural crest and placode in the guinea pig. Dev Biol 1983;96:32–36.

30. Bowman CJ, Evans M, Cummings T, et al. Developmental toxicity assessment of tanezumab, an anti-nerve growth factor monoclonal antibody, in cynomolgus monkeys (Macaca fascicularis). Reprod Toxicol 2014;53:105–118.

31. Brown MT, Herrmann DN, Goldstein M, et al. Nerve safety of tanezumab, a nerve growth factor inhibitor for pain treatment. J Neurol Sci 2014;345:139–147.