



# Anti-nerve growth factor monoclonal antibodies for the control of pain in dogs and cats

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Nerve growth factor (NGF) is essential for the survival of sensory and sympathetic neurons during development. However, in the adult, NGF and its interaction with tropomyosin receptor kinase A receptor (TrkA) has been found to play a critical role in nociception and nervous system plasticity in pain conditions. Thus, various monoclonal antibody (mAb) therapies targeting this pathway have been investigated in the development of new pharmacotherapies for chronic pain. Although none of the mAbs against NGF are yet approved for use in humans, they look very promising for the effective control of pain. Recently, species-specific anti-NGF mAbs for the management of osteoarthritis (OA)-associated pain in dogs and cats has been developed, and early clinical trials have been conducted. Anti-NGF therapy looks to be both very effective and very promising as a novel therapy against chronic pain in dogs and cats. This review outlines the mechanism of action of NGF, the role of NGF in osteoarthritis, research in rodent OA models and the current status of the development of anti-NGF mAbs in humans. Furthermore, we describe and discuss the recent development of species-specific anti-NGF mAbs for the treatment of OA-associated pain in veterinary medicine.

## Introduction

Osteoarthritis (OA) is a slowly progressive degenerative joint disease characterised by whole-joint structural changes including articular cartilage, synovium, subchondral bone and periarticular components, which can lead to pain and loss of joint function.<sup>1-4</sup> It is considered to primarily affect the hip, stifle and elbow joints in dogs,<sup>5</sup> although no comprehensive, prospective studies of the prevalence of canine OA throughout the skeleton have been performed.

Although most commonly initiated early in life by developmental disease (eg, hip dysplasia), many other factors play a role in its development, including diet, genetics, environment, obesity and age.<sup>4,6,7</sup> In cats, hip, stifle, hock and elbow joints are the most commonly affected synovial joints.<sup>3</sup> Although much less is known about the aetiology of OA in cats, idiopathic OA is considered common, with congenital, traumatic, infectious, nutritional and immune-mediated causes having been documented, similar to other species.<sup>3</sup> OA is a condition associated with clinical signs in a large percentage of the population, with an estimated minimum of 20 per cent to 30 per cent of dogs affected clinically and up to 40 per cent of all cats being affected clinically (90 per cent of all cats over 12 years of age).<sup>3,5,8</sup> The disease is currently incurable with negative consequences related to pain, mobility impairment and decreased quality of life.<sup>9</sup> Pain results in both local and distant deterioration of the musculoskeletal system as a result of decreased and altered mobility. The pathological processes of OA, such as joint capsule thickening and fibrosis, contribute to altered range of motion that compounds the musculoskeletal changes. Additionally, the ongoing nociceptive input into the CNS results in somatosensory system changes and central sensitisation<sup>10,11</sup>, which contributes to the perception of pain. The combined effects of pain, central sensitisation and activity impairment may have negative effects on

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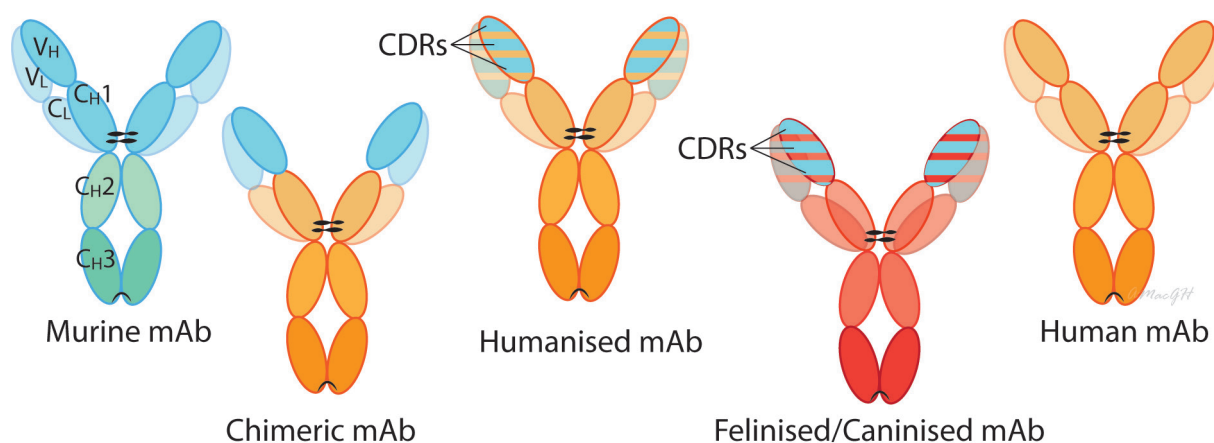
the affective state, heightening anxiety, depression, sleep impairment<sup>12</sup> and cognitive dysfunction as reported in humans.<sup>13 14</sup>

Currently, pharmacological treatment of pain centres around non-steroidal anti-inflammatory drugs (NSAIDs). These are used to relieve pain and promote functional improvement.<sup>2</sup> Globally, several NSAIDs are approved for use in dogs, but only two NSAIDs are approved for use long-term in cats and only in certain countries. Despite their widespread use and obvious benefit in many cases, NSAIDs are not always sufficiently effective when used as monotherapy.<sup>15</sup> Additionally, there are safety and tolerability concerns with their use in both dogs and cats.<sup>16–19</sup> Beyond cyclooxygenase-inhibiting NSAIDs and the recently approved piroxicam NSAID, a prostaglandin receptor antagonist, grapiprant,<sup>20</sup> treatment options for the control of pain are very limited. Furthermore, evidence for efficacy of so-called adjunctive analgesics is extremely limited<sup>15 21</sup>. Additionally, there are few proven non-drug therapies, and none that have been shown to provide rapid pain relief. Therefore, OA related-pain remains a challenging clinical entity to treat, and indeed, OA-associated pain is one of the most common reasons for euthanasia in dogs.<sup>22 23</sup> Thus, there is an urgent need to develop effective treatments for OA-related pain in dogs and cats.

### Advent of monoclonal antibodies

With greater understanding of pathogenesis of OA coupled with advances in biotechnology, specific immunomodulatory therapies called biological agents have been introduced for the treatment of joint diseases, specifically immune-mediated joint diseases.

Biological agents are medical products that are isolated from a variety of natural sources (human, animal or microorganism). This is in contrast to most drugs that are chemically synthesised. Biological agents include a wide range of products such as vaccines, blood and blood components, gene therapy and recombinant therapeutic proteins. They also may be produced by biotechnology methods. In human medicine, biological products often offer the most effective means to treat a variety of medical illnesses and conditions. The monoclonal antibodies (mAbs) have proven to be extremely effective in a variety of diseases in humans. Monoclonal antibodies are produced from single B-lymphocyte clones in mice or through recombinant engineering. They are monovalent antibodies that specifically bind to target molecules including cytokines, receptors or cells.<sup>24</sup> The binding results in blocking the activity of the target. They were first generated in mice in 1975 using a hybridoma technique<sup>25</sup> involving immunising a certain species against a specific epitope on an antigen and obtaining B-lymphocytes from the animal's lymphoid tissue. B-lymphocytes are then fused with an immortal myeloma cell, a type of B-cell tumour, and these hybridomas can be maintained in vitro and will continue to secrete antibodies with appropriate specificity. Administering purified non-human-derived antibodies to humans can cause immune reactions,<sup>26</sup> and so antibody engineering technology has been developed to lower the risk of immune reactions.<sup>27</sup> Engineering of antibodies by replacing mouse sequence-derived amino acids with human sequences has significantly reduced immunogenicity of this class of therapeutics.<sup>28</sup> Over the last 25 years, chimeric, humanised and fully human mAbs have been developed for use in humans



**Figure 1** Antibodies are large glycoproteins typically composed of two heavy chains and two light chains, each of which contain a variable domain (variable heavy (VH) or variable light (VL) and a constant domain (constant heavy (CH) or constant light (CL)). The amino acid sequence of the variable domain varies greatly among antibodies and six 'hyper-variable' complementarity-determining region (CDR) loops within the variable domains give the antibody its specificity for binding to an antigen. In contrast, the constant domain is identical in all antibodies of the same isotype but differs in antibodies of different isotypes (IgA, IgD, IgE, IgG and IgM). The tail region of the constant domain (Fc region: CH2 and CH3) may direct immune effector functions by binding to cell receptors expressed on immune cells or initiating complement-dependent cytotoxicity. Murine monoclonal antibodies (mAbs) are antibodies produced from individual cloned and immortalised mouse B cells. Most useful therapeutic antibodies have been constructed with the gamma immunoglobulin (IgG) isotype. Chimeric mAbs are antibodies made by fusing the genes encoding the variable region from a murine-derived mAb, with those from an immunoglobulin (Ig) constant region from a human antibody. Humanised mAbs retain only the CDRs (part of the variable domain from the original murine-derived mAb that binds to the specific antigen). Fully human mAbs have no murine sequences. Caninised and felinised mAbs are fully canine or feline specific. These can be made in several ways; for example, Nexvet have used a process of conversion based on alignment with immunoglobulin complementary DNA libraries (PETization).

to decrease immunogenicity<sup>29 30</sup> (figure 1). Chimeric mAbs are antibodies made by fusing the variable region from a murine-derived mAb, with the immunoglobulin (Ig) constant region from a human antibody.<sup>31 32</sup> The resulting construct is approximately three quarters human.<sup>31 32</sup> The next advance was the ‘humanization’ process.<sup>33</sup> Initially, only the complementarity-determining regions (CDRs) (part of the variable domain from the original murine-derived mAb that binds to the specific antigen) are retained, resulting in a construct that is approximately 95 per cent human.<sup>31 32</sup> Subsequently, fully human mAbs, which have no murine sequences, have been produced through transgenic mice and phage technologies.<sup>24</sup> In general, the more humanised the construct is, the less immunogenic the mAb. However, the immunogenicity cannot be predicted based only on the amount of non-human sequence on the molecule. In fact, antidrug antibodies to even fully humanised mAbs have been reported due to factors such as aggregates and adjuvant-like contaminants, although these issues have largely been resolved by improvements in manufacturing and formulating practices.<sup>34</sup> Moreover, other key factors that are relevant to the immunogenicity of a compound include route of administration (intravenous v subcutaneous), treatment paradigm (continuous v intermittent) and concurrent immunosuppressive therapy. In clinical practice, these factors have proven to be relevant considerations in the therapeutic use of mAbs.<sup>31 32</sup> Immune responses to therapeutic mAbs are undesirable as they can neutralise the action of therapeutic mAbs, and hypersensitivity can result in morbidity and mortality.<sup>35</sup> Importantly, some of the mAb immunogenicity appears to be idiotypic where patients who develop antibodies after treatment with 1 chimeric mAb might not be expected to demonstrate equal reactivity to another chimeric mAb.<sup>31 32</sup>

There are multiple mechanisms by which mAbs produce their effect. These include blockade of ligand–receptor interaction or signalling pathways; altering cell populations (by engaging effector functions including the complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity and antibody dependent phagocytosis or apoptosis).<sup>29</sup>

The activation and sensitisation of peripheral nociceptors by inflammatory and hyperalgesic mediators such as cytokines is recognised as one of the main peripheral mechanisms responsible for joint pain.<sup>36</sup> In parallel with an increased understanding of the role of the cytokines, chemokines and neurotrophins in joint pathology and pain,<sup>36</sup> there has been growing interest the use of mAb therapy to target these molecules.<sup>29</sup> Nerve growth factor (NGF) is one of the cytokines<sup>37</sup> that has received significant attention as a key regulator involved in both inflammatory and neuropathic pain.<sup>38</sup>

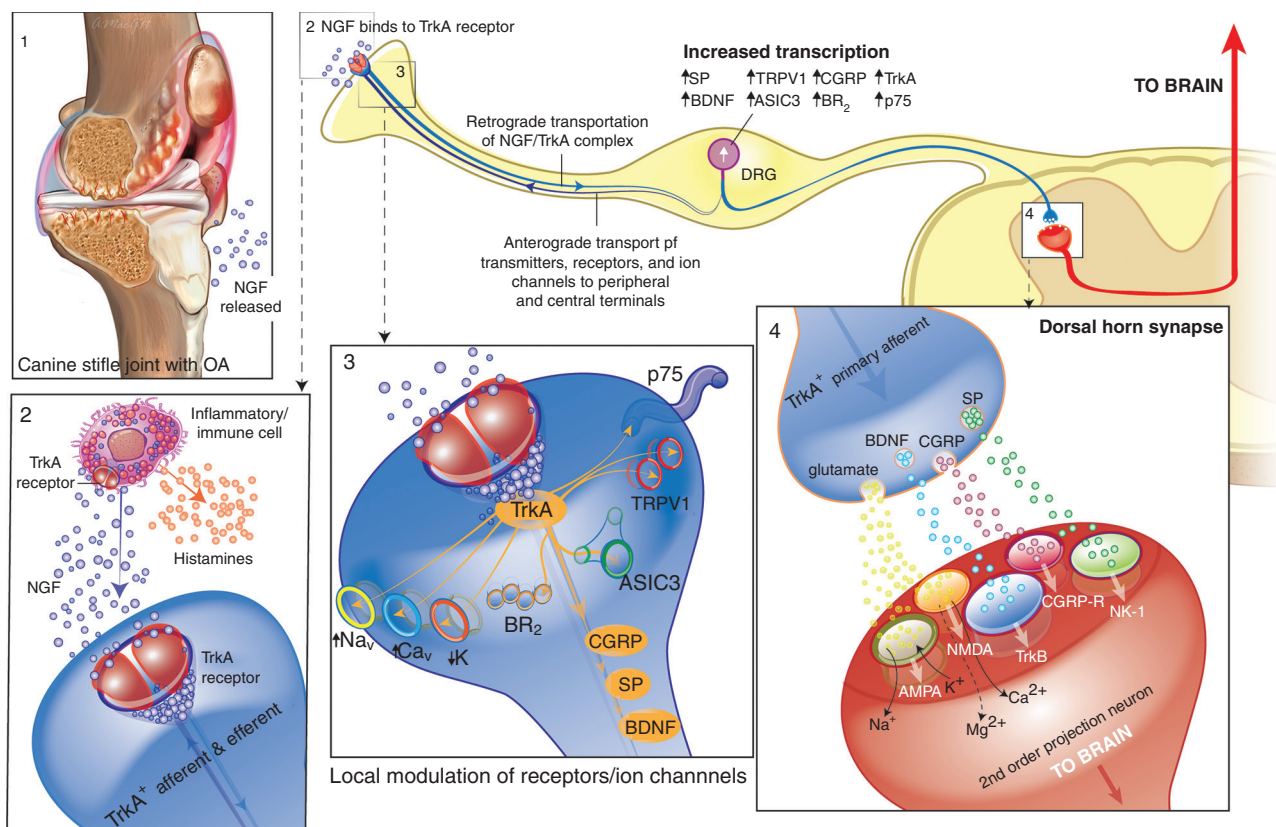
This paper will review the role of NGF in the arthritic joint, efficacy of anti-NGF therapy based on murine OA models, the current status of the development of

anti-NGF mAbs in humans and will also discuss the recent development of anti-NGF mAbs for the treatment of OA-associated pain in experimental and clinical studies in veterinary medicine.

### Monoclonal antibodies targeting NGF NGF and its pain pathway

NGF was originally discovered as a critical factor for the development and maintenance of sensory and sympathetic neurons in the developing nervous system (reviewed in ref<sup>39</sup>). In the prenatal and early postnatal periods, NGF is required for survival of both sensory and sympathetic neurons.<sup>40 41</sup> However, in adults, the main role of NGF in the periphery shifts from trophic support of sensory and sympathetic neurons to modulation of nociceptive neuronal activity.<sup>39</sup> Preclinical and clinical research over the past several decades has clearly demonstrated the important role of NGF in nociceptor sensitisation in a wide variety of both acute and chronic pain states including postoperative and OA pain<sup>39 42–44</sup> (figure 2). In this respect, NGF can be considered to be similar to prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), which also produces nociceptor sensitisation, and both play a role in the sensitisation of nerves following injury—the fundamental protective effect of pain. NGF is produced and released by peripheral tissues in response to noxious stimuli. It functions as a soluble signalling protein that mediates its activity via binding to two distinct cell surface receptors (NGFRs), the high-affinity NGF-specific tropomyosin receptor kinase A (TrkA) and the low affinity p75 neurotrophin receptor (p75<sup>NTR</sup>). When NGF binds to TrkA expressed on the peripheral terminals of sensory nerve fibres, the NGF/TrkA complex is internalised. The NGF/TrkA complex is retrogradely transported to the cell body of sensory neurons, located in the dorsal root ganglia (DRG). This modulates and/or increases the expression of a variety of cell surface receptors and ion channels involved in nociception including the transient receptor potential vanilloid 1, acid-sensing ion channels, bradykinin receptors, voltage-gated sodium channels, voltage-gated calcium channels and mechanotransducers. This results in an increase the excitability of primary afferent fibres (peripheral sensitisation) through phenotypic alterations. NGF/TrkA signalling also leads to transcriptional changes that result in the increased expression of pronociceptive neurotransmitters such as substance P, calcitonin gene-related peptide (CGRP) and brain-derived neurotrophic factor. Thus, NGF induces functional, as well as phenotypic, alterations in the primary afferent fibre. In the periphery, NGF also binds to TrkA located on mast cells and other immune cells and elicits the release of inflammatory mediators such as histamine, serotonin and NGF itself. Thus, NGF can trigger peripheral sensitisation and sensitises adjacent nociceptive neurons as a result of the release of these inflammatory mediators.<sup>39 42–44</sup> In conditions where NGF





**Figure 2** Schematic diagram of the involvement of nerve growth factor (NGF) in nociception and nervous system plasticity. In osteoarthritis (box 1), NGF is produced and released by peripheral tissues (such as chondrocytes) and can bind to its receptor, TrkA located on primary afferent (sensory) fibres. In addition, NGF that is released in the periphery also binds to TrkA located on mast cells and other immune cells and subsequently elicits the release of inflammatory mediators such as histamine, serotonin (5HT) and NGF itself (box 2). When NGF binds to TrkA, on TrkA-positive primary afferent nerve fibres, the NGF/TrkA complex is internalised and retrogradely transported to the cell body of the sensory neurons that are located in the DRG. This modulates and/or increases the expression of a variety of cell surface receptors and ion channels involved in nociception including TRPV1, ASIC, BR2, Nav, Cav, K and putative mechanotransducers, which result in an increase the excitability of primary afferent fibres (peripheral sensitisation) (box 3). NGF/TrkA signalling also leads to transcriptional changes that result in the increased expression of pronociceptive neurotransmitters such as SP, CGRP and BDNF. When these peptidergic (TrkA-positive) primary afferent neurons are subsequently stimulated, release of these peptides, in addition to glutamate acting on AMPA receptors, and binding to their respective receptors (SP to NK-1, CGRP to CGRP-R, BDNF to TrkB) may cause strong depolarisation of the postsynaptic second order projection neuron (box 4). This will result in the removal of the magnesium ( $Mg^{2+}$ ) block of the NMDA receptor, facilitating cellular windup. This increases the probability of central sensitisation and facilitated transmission through the dorsal horn synapse and then, via third-order neurons, to the sensory cortex in the brain. Thus, NGF is involved in the processes of inflammation in the periphery, and also in the sensitisation of primary afferent neurons through alteration of their functional phenotype. 5-HT, 5-hydroxytryptamine; AMPA, the  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ASIC, acid-sensing ion channel; BDNF, brain-derived neurotrophic factor; BR2, bradykinin receptor 2; Cav, voltage-gated calcium channel; CGRP, calcitonin gene-related peptide; CGRP-R, calcitonin gene-related peptide receptor; DRG, dorsal root ganglia; K, delayed-rectifier potassium channel; Nav, voltage-gated sodium channel; NMDA, the glutamatergic N-methyl-D-aspartate; NGF, nerve growth factor; NK-1, neurokinin 1 receptor; p75, neurotrophin receptor; SP, substance P; trkA, tropomyosin receptor kinase A; trkB, tropomyosin receptor kinase B; TRPV1, transient receptor potential vanilloid 1.

is playing a pivotal role in the pronociceptive processes, an analgesic that blocks NGF/TrkA signalling may be useful.<sup>44 45</sup>

It should be noted that several reports suggest that NGF and TrkA expression in the CNS may contribute to driving chronic pain.<sup>46 47</sup> The present review is focused on peripherally restricted anti-NGF mAb therapies that do not readily cross the blood–brain barrier (BBB). However, previous reports using a small molecule pan-Trk inhibitor (which readily crosses the BBB and binds to TrkA, B and C with nanomolar affinity) showed very similar efficacy as peripherally restricted anti-NGF mAb therapies in attenuating both non-malignant and malignant skeletal pain.<sup>39 48 49</sup> Thus, while peripherally restricted anti-NGF therapies presumably blocks NGF induced sensitisation and sprouting of nociceptors,<sup>39</sup> it will be important to further define how NGF and TrkA in the CNS may also contribute to the induction and maintenance of chronic pain. Additionally, it will be important to define how anti-NGF therapies that are

restricted to the periphery, such as mAbs, modulate centrally driven or maintained processes involved in chronic pain.

### NGF and OA-associated pain and pathology

Evidence for a contribution of locally produced NGF to joint pathology, as well as pain in arthritic joints has emerged. NGF can induce joint pain via a direct sensitisation of nociceptors.<sup>50</sup> Indeed, a single intra-articular (IA) injection of NGF into normal rat knees produced dose-dependent, long-lasting increases in pain behaviours (asymmetrical gait and allodynia at a site distal to the injected joint), joint swelling and synovial macrophage infiltration.<sup>51</sup> Other work has shown that in arthritic joints, NGF is released by damaged cells including synovial cells and chondrocytes, and elevated NGF and/or its receptors are detected in synovium and articular cartilage in murine models of OA.<sup>52 53</sup> Similarly, elevated NGF levels are found in synovial fluid, synovium, osteochondral junction and articular

cartilage in human patients with arthritis.<sup>54–58</sup> A recent work has shown elevated levels of NGF in synovial fluid in dogs with naturally occurring OA compared with healthy joints.<sup>38</sup> Interestingly, experimental studies have demonstrated that augmented NGF gene expression and NGF immunoreactivity in the joint correlates with pain-related behavioural changes in surgically induced OA in mice.<sup>53,59</sup> Administration of an anti-NGF/TrkA signalling molecule significantly decreased pain behaviours in a murine model of OA.<sup>59–63</sup> The antinociceptive effect of anti-NGF antibody appeared to be equal to the highest tolerated dose of indomethacin, as assessed by a vocalization test in a complete Freund's adjuvant (CFA) induced arthritic model in rats.<sup>60</sup> In another study comparing the efficacy of different analgesics, Adams *et al*<sup>61</sup> examined the ability of different analgesics (morphine, tramadol, NSAIDs and anti-NGF therapy) to attenuate gait impairment using a gait analysis system in the same arthritic model. They showed that intraperitoneal administration of anti-NGF mAb produced a profound improvement in gait parameters in a dose-dependent manner. Its effect was the same or greater than clinically efficacious doses of morphine and NSAIDs. Overall, basic science studies have shown a role of NGF in pain and pathology in OA and shown that anti-NGF therapies show robust analgesia that is equal to or greater than current analgesics.

Peripheral and central neuronal plasticity are key mechanisms in the development and maintenance of chronic pain<sup>64</sup> and evidence points to NGF as being a major determinant of plasticity in both the peripheral and CNS. Small doses of exogenous NGF delivered to intact DRG or into the intrathecal space in rats triggered a persistent hyperalgesia, indicative of peripheral and central sensitisation, respectively.<sup>65,66</sup> A recent study reported the effect of anti-NGF therapy on peripheral and CNS plasticity and associated hyperalgesia.<sup>53</sup> Behavioural tests showed significantly reduced OA-associated pain and hyperalgesia in mice injected with anti-NGF mAb compared with a saline injected control group, suggesting that anti-NGF therapy is a potentially effective analgesic treatment for both the peripheral and the central plasticity components of chronic pain. It is worth noting that reducing sensitisation by NGF signalling blockade is not anticipated to block normal, protective, nociceptive signalling unlike traditional analgesics such as opiates.<sup>67</sup>

One potential mechanism behind OA-associated pain is ectopic sprouting of sensory and sympathetic nerve fibres. With the progression of OA in humans, nerve sprouting along new blood vessels can be detected, both into structures that are normally not innervated (eg, non-calcified cartilage, the osteochondral junction and meniscus), as well as normally innervated structures (eg, synovium).<sup>53,56,57,68,69</sup> NGF is considered to be one of the main factors inducing nerve sprouting and neuromas

in response to tissue and/or nerve injury, and NGF may promote angiogenesis itself.<sup>39,56</sup> Studies of tissue from human patients with OA and rheumatoid arthritis have showed that the NGF immunoreactivity in pathological joint tissue was significantly higher than normal joint tissues. These studies showed NGF immunoreactivity was associated with increased sensory nerve innervation and sprouting (CGRP positive neurons) in the synovium and at the osteochondral junction.<sup>56,57</sup> Interestingly, data suggest NGF activity may be correlated with symptoms. Investigators compared tissue from human patients with similar macroscopic chondropathy, half of whom were symptomatic and half of whom were asymptomatic knee chondropathy patients.<sup>53,57</sup> In these reports, the NGF immunoreactivity was significantly greater in the synovium from patients with symptomatic OA compared those with asymptomatic OA.<sup>53,57</sup> Supporting this association between NGF immunoreactivity and symptoms, Kc *et al*<sup>53</sup> reported that sensory nerve (protein gene product 9.5 positive neurons) fibre innervation was markedly increased in the synovium from patients with symptomatic OA, and this change was colocalised with augmented NGF immunoreactivity. This may suggest that upregulation of sensory neurons and associated NGF/TrkA signalling are better correlated with OA symptoms than are cartilage lesions. In an experimental study in mice, sensory (CGRP and neurofilament 200 positive neurons) and sympathetic nerve fibres (tyrosine hydroxylase positive neurons) in synovium and periosteum were significantly increased following the injection of CFA.<sup>70</sup> In the same mouse model, administration of anti-NGF mAb significantly reduced the sprouting of these sensory and sympathetic nerve fibres in synovium in the OA joint and attenuated joint pain.<sup>71</sup> There appears to be good evidence that NGF may be responsible for pathological nerve sprouting in arthritis, and that this, and the presence of NGF, correlates well with clinical signs of pain.

Although NGF is known to induce angiogenesis that could contribute to inflammation, the effects of anti-NGF therapy on experimentally induced synovitis are mixed. One study reported anti-NGF therapy significantly decreased synovitis and cellular infiltration,<sup>62</sup> while other studies have concluded that anti-NGF therapy did not alter synovitis and cellular infiltration.<sup>63,71,72</sup> In clinical orthopaedics in humans and veterinary species, there is ongoing debate as to whether OA pain treatments should have anti-inflammatory effects to be optimally effective.

As cartilage damage progresses in rodent models of OA, gene expression and immunoreactivity of NGF in articular cartilage are increased.<sup>53,73</sup> Similarly, in human patients, immunoreactivity of NGF and its receptors in articular cartilage and chondrocytes are elevated with severity of cartilage damage.<sup>53,58</sup> Recently, an *in vitro* study suggested that NGF signalling is a contributing factor in articular cartilage degeneration in OA. In this

study, human cartilage tissue explants were harvested from early OA joints and cultured in serum-free medium with or without NGF for 14 days. NGF treatment resulted in extracellular matrix catabolism indicated by increased sulfated glycosaminoglycan release and matrix metalloproteinase (MMP) levels and activity.<sup>58</sup> Additionally, treatment with NGF neutralising antibody inhibited increased MMP levels and enhanced the level of tissue inhibitor of matrix metalloproteinase-1 in OA cartilage explants. However, the severity of cartilage damage in rats administered anti-NGF mAb was the same as those given saline control in a sodium monoiodoacetate (MIA) model of OA.<sup>63 72</sup> Overall, at present, it is unclear if anti-NGF therapy has any potential protective effect on articular cartilage.

Thus, given the role of NGF in nociception and contribution to OA disease progression, various ways of preventing activation of NGF/TrkA have been developed, including capturing free NGF, preventing NGF binding to TrkA or inhibition of TrkA function.<sup>39 42–44</sup> Methods for capturing free NGF and inhibition of TrkA function have been advanced into clinical trials in humans.

### Potential beneficial effects of NGF

This review focuses on the treatment of pain via inhibition of NGF. However, there are potential beneficial effects of NGF. NGF is essential for the development and phenotypic maintenance of neurons in the peripheral nervous system (PNS) and for the functional integrity of cholinergic neurons in the CNS.<sup>74</sup> In the PNS, in addition to the role in the regulation of neurotransmitters and neuropeptides synthesis, NGF has the protective action on the survival of degenerating peripheral nerve cells.<sup>39</sup> Deprivation of NGF can lead to damage of neurons, while exogenous NGF administration can promote peripheral nerve growth and re-establish functional activity.<sup>74</sup> Thus, NGF attracted clinicians for the potential clinical application to neurodegenerative diseases. However, the phase III clinical trials did not show positive results after administration of human recombinant NGF. Additionally, undesired adverse events (AEs), such as the peripheral pain, were observed.<sup>75</sup> Clinical trials were halted.<sup>74</sup> A specific role of NGF has been proposed for the cholinergic neuron population of the CNS.<sup>76</sup> NGF contributes to the maintenance of cell morphology and physiology of cholinergic neurons, which are highly dependent on NGF during both development and adulthood.<sup>77</sup> Studies demonstrated that NGF was able to promote survival of basal forebrain cholinergic neurons, known to degenerate in age-related disorders (such as in Alzheimer's disease (AD)).<sup>78</sup> This leads to the hypothesis that intracerebral administration of NGF might reduce or prevent brain neuronal degeneration of patients with AD. However, clinical studies were stopped due to only mild neurological improvements and AEs, including systemic pain.<sup>79</sup> In more recent years, other strategies have been applied for the

delivery of NGF into the damaged brain neurons and to bypass safely the BBB (eg, nose-to-brain).<sup>74</sup> NGF may affect a variety of CNS neurons, other than cholinergic origin, including the visual system.<sup>80</sup> NGF and TrkA are expressed by many tissues in the eye.<sup>81</sup> Topically applied NGF eye drops can reach the retina and the optic nerve,<sup>82</sup> and there is interest in topically applied NGF for neurodegenerative diseases, such as glaucoma and neurotrophic keratitis.<sup>83</sup> Moreover, eye NGF topical administration enhanced tear release in humans and bulldogs suffering of dry eye.<sup>74</sup> Of note in these discussions is the fact that anti-NGF mAbs are confined to the periphery because of the BBB.

Recent data have suggested that NGF is a pleiotropic factor and its actions extend beyond the nervous system.<sup>76</sup> NGF is produced and used by several cell types including structural (eg, epithelial cells and endothelial cells), accessory (eg, glial cells and astrocytes) and immune cells (eg, lymphocytes and mast cells).<sup>74</sup> During the last two decades, evidence has been accumulated supporting the hypothesis that NGF possesses potential therapeutic properties on tissue healing (cutaneous and corneal ulcers), cardiomyopathy, and myocardial ischaemia.<sup>74</sup>

### Development of anti-NGF therapy for humans

Although none of the mAbs against NGF are yet approved for use in humans, anti-NGF mAbs are in development as treatments for several pain conditions. Currently, three drugs that capture free NGF have been developed: tanezumab (humanised mAb; Pfizer, in collaboration with Eli Lilly), fulranumab (fully human mAb; Amgen) and fasinumab (fully human mAb; Regeneron Pharmaceuticals, in collaboration with Sanofi). In studies performed thus far, they have been administered intravenously or subcutaneously every four weeks to eight weeks and have demonstrated dose-dependent efficacy in human patients with moderate to severe pain associated with symptomatic knee or hip OA.<sup>84–86</sup> In a study in human patients with knee or hip OA, tanezumab reduced OA pain and improved function more than that observed with NSAIDs or opiates.<sup>87</sup> The most common AEs observed across the clinical trials performed so far were peripheral oedema, arthralgia, extremity pain and neurosensory symptoms (primarily paraesthesia, hypoesthesia and hyperaesthesia).<sup>43</sup> However, overall, the AE rate has been small (1 per cent to 10 per cent in most studies), and anti-NGF mAb therapy has been generally well tolerated by the human patients.<sup>43 88–91</sup> Abnormal sensory symptoms tended to occur within a short time after the first dose and were generally transient but tended to develop more frequently with higher doses of anti-NGF mAbs.<sup>43 84</sup> Overall, symptoms were generally considered mild to moderate in severity and did not generally result in early exit from the study.<sup>43</sup> Furthermore, most symptoms were transient and resolved without permanent



sequelae within one month.<sup>43</sup> Thus, based on work in rodent models and human clinical trials, anti-NGF mAb therapy looks promising for the effective control of OA pain. However, the development programmes for anti-NGF mAbs were temporarily put on clinical hold by the Food and Drug Administration (FDA) due to an increased incidence of serious joint-related AEs from 2010 to 2012. The incidence of serious joint-related AEs were initially postulated to be osteonecrosis and/or rapidly progressive OA (RPOA) in the hip, knee and shoulder joints, which lead to early than expected joint replacement. These AEs were reported in 83 patients who had either received tanezumab monotherapy or tanezumab and NSAIDs. The incidence rate was 9 and 23.9 per 1000 patient-years (the sum of events divided by the duration of administration of tanezumab), respectively. These AEs occurred during phase II and III trials in the tanezumab development programme where the mean duration of treatment was 199 days.<sup>92</sup> The incidence of joint destruction was higher in the patients with longer exposure of anti-NGF mAbs, larger doses of anti-NGF mAbs and concurrent use of NSAIDs.<sup>42 92</sup> However, serious joint-related AEs were observed in some patients following a single treatment of anti-NGF mAb.<sup>91</sup> Several cases occurred in multiple joints and also in non-index joints.<sup>92</sup> Following extensive adjudication of these AEs,<sup>92</sup> less than 1 per cent of the AEs were deemed to be due to osteonecrosis and the majority classified as RPOA. Characteristics of RPOA are rapid clinical deterioration (increase in pain) and radiographic progression of joint degeneration.<sup>93</sup> The cause of these cases of RPOA is not currently understood and experimental studies found no evidence of a direct adverse effect on bone healing or joint health (bone, cartilage, joint vasculature or joint innervation) in animals (rodents) treated with anti-NGF mAbs at large multiples of the clinical exposure.<sup>94 95</sup> In human medicine, the most prominent concern around anti-NGF revolves around RPOA. Although theories have been proposed, the cause of anti-NGF related RPOA remains unclear.<sup>96</sup> Overloading, resulting from increased activity and weight-bearing due to good analgesia (analgesic arthropathy), immune reactions and neuropathic arthropathy (nerve damage resulting in loss of ability to feel the joint and decreases in joint stability) have been suggested as potential factors leading to RPOA following anti-NGF therapy.<sup>91 97 98</sup> The increased incidence of RPOA associated with concurrent NSAID use is not understood, but it is possible that NSAIDs contribute to RPOA through prostaglandin-dependent and prostaglandin-independent mechanisms, including increasing the risk of microvascular thrombotic events in bone and inhibiting the repair of subchondral microfractures.<sup>91</sup> However, this is speculative at the moment. To the authors' best knowledge, the influence of coadministration of anti-NGF therapy and NSAIDs on joint health has not yet been evaluated in basic

studies, and the reason for the increased incidence of RPOA associated with concurrent use of anti-NGF mAb and NSAIDs has not been elucidated. It appears the recommendation will be to avoid use of NSAIDs concurrently with anti-NGF therapy (see below). Another concern with using anti-NGF mAb was whether this therapy could cause loss of sensory or sympathetic nerve fibres in the adult as NGF itself is known to be required for the normal development of sensory and sympathetic nerve fibres in developing animals and humans. Experiments in both mice and monkeys showed that anti-NGF therapy did not induce loss of sensory or sympathetic nerve fibres in the skin or bone nor any sign of injury or degeneration in the cell bodies of sensory neurons in the DRG.<sup>94 99 100</sup> Interestingly a partial FDA clinical hold was placed on all anti-NGF programmes from 2012 to 2015 due to anatomical changes in the cell bodies of postganglionic neurons, which was based on an observed reduction in size and neuronal count of primates receiving very high doses of anti-NGF therapy. However, subsequent detailed toxicology studies in monkeys did not demonstrate any reduction in sympathetic function or neuronal cell counts.<sup>89 101</sup> While it did appear that primates exposed to high and prolonged doses of anti-NGF did have a reduced size of postganglionic sympathetic cell bodies, this change in cell body size returned to normal upon cessation of anti-NGF administration.<sup>100</sup> In light of these data, a risk minimisation plan has been incorporated subsequent anti-NGF human studies by excluding patients with ongoing disorders of the sympathetic nervous system.<sup>100</sup>

After reviewing the data, the FDA advisory committee concluded that these serious joint AEs were probably related to the anti-NGF treatment. However, clinical trials for the development of anti-NGF mAbs in human have been restarted in 2015 with the adoption of a risk mitigation strategy, which included monitoring patient overuse of the skeletal using accelerometers, dosing restrictions and a recommendation against concomitant NSAID use in patients with OA. The decision by the FDA to allow the continued development of the drug class was made due to the potential significant benefit of anti-NGF therapy for a multitude of pain conditions and the absence of any direct link between the administration of anti-NGF mAbs and joint destruction. Indeed, in 2017, tanezumab received FDA fast track designation, recognising the significant potential benefit from this therapeutic. A recent search of the National Institutes of Health web site (ClinicalTrials.gov) revealed that tanezumab is currently in phase III studies in the patients with OA of the hip and knee and chronic lower back pain with the bone cancer pain trials being conducted outside the USA.<sup>90</sup>

Glenmark Pharmaceuticals Ltd. has developed a TrkA antagonist (GBR 900) with the target indication being the treatment of chronic pain. This antibody has completed phase I enabling preclinical development

programme<sup>102</sup> and a phase I trial in normal volunteers (ClinicalTrials.gov identifier: NCT02235727). In the Good laboratory practice (GLP) toxicity studies, no dose-limiting toxicities were detected with GBR 900, even at high doses. This potentially differentiates GBR 900 from anti-NGF antibodies that do show preclinical toxicity. Reportedly, preclinical head-to-head comparisons with anti-NGF antibodies in animal models of inflammatory pain demonstrated that GBR 900's efficacy profile compares favourably with that of anti-NGF antibodies.<sup>102</sup> Another TrkA inhibitor (GZ389988) has been developed by Sanofi, formulated for IA administration to control OA pain. In the rat MIA model, a single IA injection of GZ389988 resulted in more normal weight-bearing for four weeks without any significant histopathological changes in joint tissues compared with placebo.<sup>103</sup> Interestingly, IA injection into the contralateral joint had no effect on the ipsilateral limb joint pain, suggesting that IA injection does not result in substantial systemic exposure, which may limit the risk of AEs. A proof-of-concept study to assess the efficacy, tolerability and safety of GZ389988 in human patients with painful OA of the knee has recently completed, but results are not yet available (ClinicalTrials.gov identifier: NCT02845271).

#### Development of anti-NGF mAbs for the dog and cat

Canine and feline NGF are closely homogenous to NGF in other species, such as human and mice. However, as mAbs from one species often can induce an immune response when used without modification in another species, for therapeutic purposes, antibodies need to be species specific to reduce the risk of immunoreactions to the antibody. It is likely that several companies have developed or are developing technology to create species-specific antibodies for the veterinary market (as evidenced by the recent approval of Zoetis' interleukin (IL)-31 mAb (<https://www.zoetisus.com/products/dogs/cytopoint/>)), but in the pain arena, the only publicly available information at the time of writing this review was for the company Nexvet, which was recently purchased by Zoetis (<http://news.zoetis.com/press-release/investors/zoetis-acquire-nexvet-biopharma-innovator-mono-clonal-antibody-therapies-comp>). Nexvet developed technology to create anti-NGF mAbs specifically for canine and feline use. In this approach, complementary DNA libraries are used to compare the natural variations in the sequences of the heavy and light chain of the mAb between donor species (human or rodent) and target species. This comparison enables the determination of the minimal number of changes at each position in the amino acid sequences that are required to convert the donor mAb variable region heavy and light chain sequences into mAb sequences containing only amino acids identified within target species. At sites where amino acid changes

are necessary, the most similar amino acid in the matrix of the target species is substituted.

This results in 100 per cent species-specific mAb sequences that carry a lower risk of rejection due to immunoreaction, while preserving high affinity and potent bioactivity. Nexvet successfully converted the rat anti-NGF mAb ( $\alpha$ D11) into caninised and felinised anti-NGF mAbs with the goal of managing pain states, including OA.

#### Development of anti-NGF therapy for canine OA

Early work with Nexvet's fully caninised anti-NGF mAb (ranevetmab) indicated a favourable PK profile (mean tissue distribution phase half-life of approximately 12 hours and a mean plasma half-life time of nine days) and no evidence of an acute neutralising immunogenic response in dogs.<sup>104</sup> In the kaolin injection pain model in dogs, efficacy was seen (reduced lameness).<sup>104</sup> Currently, there are two published clinical trials that evaluate the efficacy of single intravenous injection of ranevetmab (0.2 mg/kg).<sup>105 106</sup> Both studies required a two-week withdrawal period of NSAIDs prior to the study starting, and NSAIDs were not permitted to be used throughout the study period, in order to best assess efficacy of the anti-NGF therapy. In a randomised and double-blind study where all dogs received ranevetmab, the safety and clinical effect was examined using an owner completed questionnaire—the Canine Brief Pain Inventory (CBPI) score.<sup>105</sup> Nine dogs with OA received a single injection of ranevetmab during the 10 weeks of study period (either at the start, two or four weeks into the study), with owners blinded to the time of injection. They were evaluated every two weeks for six weeks following injection. At other evaluation times, the dogs received sterile saline, and the owners were unaware of the evaluation time at which the mAb had been administered. This study showed that significantly lower CBPI scores were seen compared with baseline scores until four weeks after treatment, and although values were not statistically significant, CBPI scores at six weeks after administration were still lower than baseline scores. In another randomised, double-blind, placebo-controlled pilot study,<sup>106</sup> 26 dogs suffering from OA pain were allocated to placebo or treatment group based on predominant site of problem (fore or hind limb impairment) and CBPI score. The dogs were assessed every two weeks for four weeks using objective accelerometry (which measures activity and movement) and subjective, owner-completed clinical metrology instruments (CMIs, CBPI, Client-Specific Outcome Measures (CSOM) and Liverpool Osteoarthritis in Dogs) for the evaluation of efficacy. The dogs that received ranevetmab had significant improvement in all three CMIs compared with baseline scores throughout the study period and significantly greater activity compared with placebo group during the daytime period (09.00–17.00). Additionally, the



distribution of success/failure rates for the CBPI and CSOM was very similar to previous studies using carprofen and grapiprant.<sup>20 107</sup> In both clinical studies, no AEs associated with treatment were reported. Finally, although the data have not been published yet, a pivotal, multicentre, placebo-controlled, randomised, double blind study was conducted at 12 sites (<http://ir.nexvet.com/phoenix.zhtml?c=253841&p=irol-newsArticle&ID=2176442>: this was announced on the website, but it is no longer active). Two hundred and sixty-two dogs with OA were enrolled in this study and allocated to treatment or placebo on a 2:1 ratio. They received ranvetmab subcutaneously once per month for three months. The efficacy and safety of the drug were evaluated using CMI (CBPI and CSOM) over 84 days. Subcutaneous administration of ranvetmab appeared to result in a statistically significant improvement on the assessed level of pain as measured using CBPI improvement success/fail criteria as well as using CSOM improvement success/fail criteria. Furthermore, subjective veterinarian assessment of limb function and joint pain were also numerically superior to placebo for each composite variable at most evaluation points and included a statistically significant overall treatment effect for lameness, particularly relevant given the large placebo effect seen in veterinarian lameness assessments.<sup>108</sup>

Basic science studies have evaluated the IA route of administration, but as yet, there are no reports of studies looking at IA injection in the dog. Although there are no other publications on anti-NGF mAbs and dogs, a recent search revealed that Zoetis LLC<sup>109</sup> and Abbott Laboratories<sup>110</sup> as well as Nexvet Biopharma<sup>111-114</sup> hold patents for anti-NGF mAb in dogs.

### Development of anti-NGF therapy for feline OA

Currently, to our knowledge, Abbott Laboratories<sup>110</sup> and Nexvet Biopharma<sup>115</sup> hold a patent for anti-NGF mAbs in cats. However, the only published data in cats are for the Nexvet feline anti-NGF mAb.

Initial pharmacokinetic and efficacy evaluation of frunvetmab, Nexvet's fully feline anti-NGF mAb, were performed by investigators using eight cats administered four different doses (from 2.0 mg/kg to 28 mg/kg) subcutaneously with plasma concentrations measured over a 42-day period following injection.<sup>116</sup> Frunvetmab had a peak plasma concentration of approximately three days and a mean plasma half-life time of nine days. It was well tolerated at dosages up to 28 mg/kg. In placebo-controlled, unblinded work, using the kaolin injection pain model of cats showed that a single dose of 2 mg/kg significantly decreased subjective lameness scores compared with placebo treatment.<sup>116</sup> No AEs were seen. A single clinical trial has been published.<sup>117</sup> This was a blinded, placebo-controlled pilot study conducted in 34 cats with OA-associated pain to

assess the efficacy of a single dose of frunvetmab (0.4 mg/kg or 0.8 mg/kg, subcutaneously) over a nine-week period. A two-week wash-out period from NSAIDs was required prior to the study, and cats were not permitted to be on NSAIDs during the study period. Outcome measures were objectively measured using collar-mounted accelerometers and CMIs.<sup>117</sup> Objective accelerometry revealed a significant increase in activity compared with placebo treatment from two weeks to six weeks after injection. Furthermore, the mean activity increase over placebo over first three weeks after treatment (12.9 per cent) was greater than the increase over placebo produced by daily administration of 0.035 mg/kg of meloxicam (5.97 per cent) over a three-week period in an earlier study.<sup>118</sup> Subjective owner assessments showed a significant effect of treatment over the first three weeks after administration, the first time in the published literature that owners have been able to correctly distinguish between treatment and placebo in cats with OA-associated pain under a parallel design, placebo-controlled study. This is particularly noteworthy given the very high caregiver placebo effect seen in feline chronic pain studies.<sup>119</sup> The anti-NGF mAb was well tolerated, with a single injection of frunvetmab producing positive treatment effects with a duration of up to six weeks. Although the data have not been published yet, multicentre, placebo-controlled, randomised, double-blind pilot field study that enrolled 126 cats with OA was conducted over three months to examine the efficacy of intravenous and subcutaneous monthly administration of frunvetmab (<http://ir.nexvet.com/phoenix.zhtml?c=253841&p=irol-newsArticle&ID=2176442>: this was announced on the website, but it is no longer active). It appeared that both routes of administration were effective and, when they were combined for analysis, frunvetmab demonstrated statistically significant efficacy over placebo at multiple time points using CMIs (CSOM and Feline Musculoskeletal Pain Index). No significant adverse safety signals were observed in this study. In December 2016, Nexvet announced initiation of pivotal efficacy study of frunvetmab in cats: a placebo-controlled, randomised, double blind study with a target enrolment of 250 cats with OA at approximately 20 clinical sites around the USA. Enrolled cats will be randomly assigned to receive frunvetmab or placebo at a 2:1 ratio. Each cat will receive three doses, with each dose given 28 days apart. There are no updates on this at the time of publication.

Generally, anti-NGF mAb exposure is limited to peripheral tissues, because mAbs do not cross the BBB,<sup>120</sup> although recent work has been performed developing techniques to allow mAbs to cross the BBB.<sup>121</sup> However, anti-NGF mAbs can pass through the placental blood barrier and also be excreted in

milk,<sup>122</sup> and their use should be avoided in pregnant or lactating animals. Anti-NGF mAbs from the maternal circulation cause fetal abnormalities in rodents, and in pregnant non-human primates, they caused increased rates of stillbirth, and increased postpartum infant mortality and morbidity, decreased infant growth, sensory and sympathetic nerve system changes and decreased infant primary antibody responses.<sup>123</sup>

### **Current limitations of our knowledge of anti-NGF therapy for canine and feline OA**

Overall, in both dogs and cats, significant improvement has been seen in both subjective and objective (activity) measures following administration of anti-NGF mAbs, suggesting a positive analgesic effect of the same magnitude or greater than that expected with NSAIDs. However, the current assessment of the response to anti-NGF mAbs has not included gait analysis, which was the mainstay of assessment of NSAIDs until recently. Gait analysis is most suited to dogs with asymmetrical gait, and the dogs recruited to the studies performed thus far had multiple joint OA, better reflecting the majority of dogs with OA.<sup>106</sup> This is why gait analysis was not used, and an alternative objective measure, activity monitoring, was used. However, it would be interesting to know how anti-NGF therapy compares with NSAIDs using gait analysis in dogs, as well as in cats.<sup>124-126</sup> Although not yet performed, Quantitative Sensory Testing<sup>10 11 127</sup> could be used to more completely assess sensory function changes, as the most common AEs in human use is abnormal peripheral sensation.<sup>43</sup>

While no anti-NGF therapy-related AEs in dogs and cats have been seen, the safety of long-term exposure over years, possibly starting early in life, needs to be determined. Currently, the longest follow-up time after anti-NGF mAb therapy in dogs and cats is three months, compared with the mean trial duration of 199 days in humans. Additionally, the safety of concomitant use of NSAIDs with anti-NGF mAb therapy has not been elucidated. This will be important information because in addition to higher dose and longer exposure to anti-NGF mAbs, concurrent use of NSAIDs was a risk factor for the development of RPOA in the human trials.<sup>42 92</sup> Interestingly, RPOA is not a recognised or described phenomenon in dogs or cats, anecdotally or otherwise. Additionally, the normal rates of progression of radiographic OA in veterinary species has not been defined, leaving no 'benchmark' against which to evaluate potential cases of RPOA. Such a benchmark will be important in order to distinguish clinicians' focus on the progression of OA in cases that receive an anti-NGF mAb from true RPOA. Currently, there are no data to inform whether NSAIDs can be used with anti-NGF mAbs, nor what sort of withdrawal period (if any) is needed for NSAIDs prior to animals receiving anti-NGF mAbs.

It is likely that if approved, anti-NGF therapy will be used in joint diseases of differing aetiopathogenesis, and there is much to learn about the role of NGF and the effects of anti-NGF in relation to the differing pathologies, such as immune-mediated joint disease versus non-inflammatory OA.

One final consideration is that if the pain relief is indeed greater than seen with NSAIDs, it may be prudent to control the increase in activity seen after administration of the anti-NGF mAb in order to prevent musculoskeletal damage due to overuse of a poorly conditioned body. This is speculative but something that should be considered as clinical use begins.

### **Potential use of anti-NGF in other pain conditions**

Endogenous levels of NGF are increased in a wide range of painful disorders such as inflammatory arthritis, degenerative intervertebral disc disease, prostatitis and cancer. Administration of anti-NGF mAb has been shown to provide effective analgesia in a number of animal models of human disease including inflammatory arthritis, fracture pain, joint surgery, cancer pain and pancreatic pain.<sup>71 94 128-130</sup> Interestingly, in nearly all of conditions, anti-NGF on average reduced pain by 30 per cent to 50 per cent and this anti-NGF reduction of pain does not appear to dissipate with time of treatment.

### **Cancer pain**

An estimated 30 per cent to 50 per cent of human patients with cancer experience moderate to severe cancer-related pain, and in advanced or metastatic cancer, 75 per cent to 95 per cent of human patients report life-altering cancer induced pain.<sup>131</sup> Although pain arises from numerous causes, bone metastasis is the most common cause of cancer pain. This occurs in 60 per cent to 84 per cent of patients.<sup>132 133</sup> However, therapeutics such as opioids, which are commonly used in these patients, are not fully effective in many patients and often have significant side effects. In addition, the use of opioids is contributing to the opioid epidemic.<sup>134</sup> Thus, in humans, a significant unmet need remains for development of novel agents for cancer pain treatments. Preclinical and clinical research over the past several decades have suggested that anti-NGF mAb therapy might have an impact on the management of cancer pain.<sup>90 135 136</sup> Almost all sensory innervation of bone is provided by nociceptors that express CGRP and TrkA, which are susceptible to the blockade by anti-NGF therapy.<sup>128</sup> Additionally, cancers express TrkA and p75<sup>NTR</sup> and are stimulated by NGF, which is thought to contribute to cancer progression.<sup>137</sup>

Rodent models suggest significant therapeutic potential in cancer pain in humans, and the same is likely true of companion animals. To our knowledge, no efficacy studies with anti-NGF mAb have been

performed. However, a study showed that the majority of canine osteosarcoma primary tumours and pulmonary metastases expressed TrkA protein, and its signalling protected against apoptosis since blockade of NGF/TrkA signalling induced apoptosis of canine osteosarcoma cell lines.<sup>138</sup> Thus, anti-NGF mAb may provide significant relief of bone cancer pain<sup>90</sup> and slow the progression rate of tumour growth.<sup>137</sup>

### Other orthopaedic pain conditions (fracture and joint surgery)

Several rodent studies using hind limb fracture models (femur and tibia) in mice demonstrate the analgesic potential of anti-NGF therapy. In these models in mice, anti-NGF or anti-TrkA mAbs were administered intraperitoneally following establishment of the fracture, and pain was assessed using observational pain behaviour, ground reaction forces, mechanical allodynia and recording of locomotor activity. The treatment with both anti-NGF and anti-TrkA mAbs significantly reduced pain-related behaviour.<sup>70 94 95 139</sup> Furthermore, the efficacy of anti-NGF mAb therapy in reducing pain-related behaviours was comparable with or greater than that achieved by subcutaneous administration of therapeutic dose of morphine.<sup>70</sup> In these studies, three to four weeks after fracture, bone healing was assessed by various techniques such as mechanical testing, histomorphometric analysis, microcomputed tomography and radiographical analysis. They concluded that blockade of NGF/TrkA signalling does not influence fracture healing when compared with vehicle control group.

In a mouse model of knee joint surgery pain (drilling and coring the trochlear groove of the femur), pre-emptive intraperitoneal administration of anti-NGF mAb significantly attenuated reduction of spontaneous activity and frequency of vertical rearing following surgery compared with vehicle treated mice.<sup>130</sup>

Such work suggests potential for the use of anti-NGF mAbs for perioperative orthopaedic pain, and such therapy would be welcome in veterinary medicine where proven analgesic efficacy that can be provided following discharge from the clinic is very limited. However, much work is needed to define any potential side effects related to healing or reinnervation of surgical sites.

### Visceral pain

Tanezumab has been studied in two proof-of-concept trials involving chronic visceral pain in humans.<sup>140</sup> Patients with interstitial cystitis were administered a single dose of intravenous tanezumab, a human anti-NGF mAb, 200 µg/kg (n=34) or placebo (n=30), and assessed over 16 weeks using questionnaires.<sup>140</sup> Tanezumab provided a statistically significant reduction in pain scores compared with placebo over the first six weeks and reduced urgency frequency. In

a study evaluating patients with moderate-to-severe chronic prostatitis/chronic pelvic pain syndrome, 30 patients received a single intravenous dose of tanezumab (20 mg) and 32 received a placebo.<sup>141</sup> They were followed over 16 weeks after injection. Although average pain score and urgency episode frequency trended downwards at a six-week follow-up, neither outcome achieved significance. Anti-NGF therapy may have potential in visceral pain syndromes; however, visceral pain syndromes are not yet well defined in companion animals, except for interstitial cystitis in cats.

The main symptom of patients with pancreatitis is severe abdominal pain. NGF was found to be upregulated and notably expressed in the pancreas in both human patients with chronic pancreatitis and a rat model of acute and chronic pancreatitis.<sup>142 143</sup> Furthermore, in the study of patients with chronic pancreatitis, the expression levels of NGF and TrkA mRNA in pancreas were strongly related with pain intensity and frequency.<sup>142</sup> Although, to the best of our knowledge, no clinical study has been conducted, several studies in acute or chronic pancreatitis models in rats showed robust reduction of pain following an administration of blockade of NGF/TrkA signalling.<sup>129 144 145</sup> Anti-NGF therapy may be useful for the pain management of acute or chronic pancreatitis in companion animals.

### Conclusion

Overall, the rationale for using anti-NGF therapy in several pain conditions is strong. Current evidence indicates that anti-NGF mAb therapy has positive analgesic effects and is well tolerated for up to three months in dogs and cats suffering from OA-associated pain. Additionally, the efficacy of single injection appears to last at least four to six weeks and the magnitude of effect appears the same as, or greater than, that expected with NSAIDs. Thus, anti-NGF mAb therapy could be an alternative to the pharmacological pain management options currently available. Of particular relevance at the moment is the fact that anti-NGF therapy is a non-opioid analgesic. Further studies are needed to better understand the level of analgesic effect and the duration, and also what potential AEs and immunogenicity may occur.

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