Antagonism of Nerve Growth Factor-TrkA Signaling and the Relief of Pain

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ABSTRACT

Nerve growth factor (NGF) was originally discovered as a neurotrophic factor essential for the survival of sensory and sympathetic neurons during development. However, in the adult NGF has been found to play an important role in nociceptor sensitization after tissue injury. The authors outline mechanisms by which NGF activation of its cognate receptor, tropomyosin-related kinase A receptor, regulates a host of ion channels, receptors, and signaling molecules to enhance acute and chronic pain. The authors also document that peripherally restricted antagonism of NGF-tropomyosin-related kinase A receptor signaling is effective for controlling human pain while appearing to maintain normal nociceptor function. Understanding whether there are any unexpected adverse events and how humans may change their behavior and use of the injured/degenerating tissue after significant pain relief without sedation will be required to fully appreciate the patient populations that may benefit from these therapies targeting NGF.

PAIN is essential for the protective sensibility that enables the avoidance of tissue injury and promotes healing after injury. However, many types of chronic pain become more of a burden than benefit because they have a significant, negative impact on functional status and quality of life, and in some cases become more of a burden than benefit because they have a significant, negative impact on functional status and quality of life, and in some cases continue more of a burden than benefit because they have a significant, negative impact on functional status and quality of life, and in some cases...
of life. Persistent chronic inflammatory, neuropathic, and cancer pain present major health challenges throughout the world.1,2 However, management of chronic pain is often ineffective or incomplete3–5 because current therapies are far from ideal, attributable in part to a high incidence of dose-limiting side effects.4,5 Indeed, few current treatments effectively control chronic pain without unwanted side effects and/or abuse liability.

International guidelines recommend a multimodal combination of pharmacologic and nonpharmacologic modalities as the most effective strategy for managing chronic pain and its associated disabilities; the goal of treatment should be to effectively reduce pain and suffering while improving function.6

The effective management of chronic pain can improve patients’ quality of life, functional status, and reduce healthcare costs.5,11 However, despite significant advances in our understanding of the pathophysiology of chronic pain,12 its management continues to challenge physicians.5 The development of new agents for managing chronic pain without significant cardiovascular, gastrointestinal, or central nervous system side effects remains a significant, unmet clinical need.

In the current article, we present evidence for a new approach to the management of chronic pain that targets the effects elicited by nerve growth factor (NGF). The major objectives of this article are to review the science behind targeting NGF or its cognate receptor tropomyosin-related kinase A receptor (TrkA) for the relief of pain, outline the preclinical and clinical data suggesting that these therapies may be efficacious for relieving several types of chronic pain, and discuss potential side effects of these therapies. For more detailed and exhaustive scientific discussion of NGF and its receptors, there are several excellent reviews.

NGF Belongs to a Family of Neurotrophins

Nerve growth factor belongs to a family of molecules known as neurotrophins, which are approximately 12.5-kd proteins that form tightly bound homodimers. The neurotrophin family of target-derived proteins regulates the survival, development, and function of subsets of sensory and sympathetic neurons.17,18 Other mammalian members of the neurotrophin family are brain-derived neurotrophic factor (BDNF), neurotrophin-3 and neurotrophin-4/5. The specificity of action of these molecules is a result of their binding specificity to a family of receptors called tropomyosin-related kinase (Trk) receptors.19 TrkA preferentially binds NGF; TrkB binds both BDNF and neurotrophin-4/5; and TrkC binds neurotrophin-3. Neurotrophins also signal via a second receptor called the p75 receptor, which binds all neurotrophins (i.e., there is little specificity exerted via the p75 receptor). Trk receptors often are referred to as high-affinity receptors, in contrast to the low affinity p75 receptor. However, the difference between Trk and p75 receptors is not one of affinity but rather kinetics.

NGF binds to TrkA, whereupon the NGF-TrkA complex is internalized and transported from peripheral terminals to sensory cell bodies in the dorsal root ganglion (DRG).20–22 Evidence from several sources suggests that NGF cannot initiate signaling in the cell soma and that instead the NGF-TrkA complex activates transcription factors that control downstream gene expression.21,23 Interactions between p75 and TrkA receptors in determining the response to NGF have been reported.24,25 Furthermore, there is evidence that NGF and BDNF can sensitize the discharge of sensory neurons through p75 receptors.26,27 However, because this review is directed toward the effects of NGF in enhancing acute and chronic pain in the adult, and Trk antagonists also produce significant relief of chronic pain, in this review we focus on the NGF-TrkA system.

The NGF-TrkA Nociceptor Axis: From Development to Adulthood

The role of NGF in neuronal development has been known since its discovery nearly 60 yr ago.28 NGF plays a critical role in the development of the peripheral nervous system by promoting growth and survival of some neural crest-derived cells in developing embryos, in particular sensory and sympathetic neurons.28,29 An important documentation of these relationships is that selective mutations in NGF or TrkA genes cause congenital insensitivity to pain in humans and loss of pain behaviors in genetically altered mice.30–34 For example, congenital insensitivity to pain with anhidrosis, a human condition in which patients generally have normal proprioception and normal sensation to innocuous pressure but abnormal sensation to thermal stimuli, is caused by a mutation in the TrkA gene35 that results in a structural neuropathy affecting unmyelinated peripheral nerve fibers. Indeed, genetically modified animals lacking the NGF or TrkA gene are born with virtually no small-caliber primary sensory neurons and are profoundly unresponsive to noxious stimuli.19,32,33

Studies of NGF deprivation during critical periods of growth support the results of these genetic manipulation experiments. One method of producing long-term NGF deprivation is by immunizing animals to induce autoimmunity against NGF. Such studies have reported that NGF is in-
volved in maintenance of sympathetic neurons and the regula-
tion of the substance P (SP) content of embryonic and
neonatal sensory neurons.\textsuperscript{36,37} Immunizing pregnant rats
against NGF causes depletion of SP in DRG neurons in
animals exposed \textit{in utero} or as newborns,\textsuperscript{38,39} although the
regenerative capacity of DRG neurons after axotomy in
NGF-immunized animals was unimpaired.\textsuperscript{37} Anti-NGF an-
tibody administered during early postnatal development in
rats has revealed that DRG neurons lose the requirement for
NGF for survival shortly after birth, but NGF still has an
influence on the phenotype of nociceptors for another 10 d.
This was shown by demonstrating that withdrawal of NGF
during a critical period led to a developmental switch of
high-threshold mechanoreceptors to sensitive mechanore-
ceptors, which normally are relatively rare.\textsuperscript{40} Importantly,
this phenotypic switch of nociceptors occurs in the absence
of cell death, despite the loss of NGF.\textsuperscript{41}

Collectively, immunologic and genetic studies of NGF de-
privation during development and maturation demonstrate
that NGF has three separate roles—one for survival and de-
velopment of sensory and sympathetic neurons, the second in
maintaining the peptidergic phenotype of primary afferent neu-
rons in the early postnatal period, and the third being a key
upstream modulator of the expression and sensitization of a
variety of neurotransmitter, receptor, and ion channels ex-
pressed by adult nociceptors. However, whether adult sensory
neurons require NGF for maintenance of their phenotype and,
if so, how much NGF remains to be determined.

NGF-TrkA Signaling, Nociceptors, and Pain
in the Adult

\textbf{A Role for NGF in Nociception in the Adult}

A role for NGF has been demonstrated in acute, transient
nociceptive responses and in longer-term, chronic pain.\textsuperscript{42–45}
As early as 1977, a report that NGF exerts effects on mast
cells suggested that the physiologic effects of NGF were not
limited to neuronal development and maturation.\textsuperscript{42} The in-
volve ment of NGF in nociception and the ability of NGF to
sensitize nociceptors occurs only after sensory fibers have lost
their dependence on NGF for survival.\textsuperscript{46} As we discuss be-
down, the NGF-TrkA axis appears to play a pivotal role in the
early, intermediate, and long-term generation and mainte-
nance of several types of acute and chronic pain.

An important point in assessing the involvement of the
NGF/TrkA pathway in driving a particular chronic pain
state is the issue of the specific populations of primary affer-
ent sensory nerve fibers that innervate the injured/diseased
tissue. Four broad subtypes of primary sensory neurons have
been characterized within the DRG, of which three broad
categories are known to be important in nociceptive trans-
mission in the normal animal: thin myelinated \( \Delta \)-fibers,
peptidergic unmyelinated (C-) fibers, and nonpeptidergic
unmyelinated (C-) fibers.\textsuperscript{37} Peptidergic C-fibers and the ma-
dority of \( \Delta \)-fibers express TrkA, corresponding to approxi-
mately 40\% of adult DRG cells,\textsuperscript{48} and are responsive to
NGF.\textsuperscript{47,48} These TrkA-positive fibers innervate skin, viscera,
muscle, and bone.\textsuperscript{49–52} In contrast, nonpeptidergic C-fibers
(which express c-RET or the binding site for the lectin \textit{Grif-
fonia simplicifolia} IB4) lack TrkA or p75\( \text{NTR} \) and thus are un-
responsive to NGF (TrkA-negative); these fibers innervate skin
but not the skeleton.\textsuperscript{52–54} These data suggest that a key factor to
consider when assessing the analgesic efficacy of targeting NGF-
TrkA signaling in an acute or chronic pain state is the fraction of
NGF-responsive (TrkA-positive) nociceptors that innervate the
tissue from which the pain is arising because this innervation,
and thus the analgesic efficacy of targeting NGF-TrkA signal-
ing, may vary considerably from tissue to tissue.

\textbf{Direct Actions of NGF}

The pivotal role of NGF in inflammatory pain is exemplified
by the expression and/or release of NGF by certain inflam-
atory cells, including eosinophils, lymphocytes, macro-
phages,\textsuperscript{55,56} and mast cells,\textsuperscript{57} as a consequence of injury (fig. 1).
Moreover, NGF is up-regulated in experimental models
of inflammation, including those induced by carrageenan,
formalin, and complete Freund’s adjuvant,\textsuperscript{45,58–60} as well as
in models of autoimmune arthritis\textsuperscript{61} and ultraviolet-B-radi-
ation–induced acute inflammation.\textsuperscript{62} Cutaneous adminis-
tration of NGF to rodents\textsuperscript{63} and humans\textsuperscript{64} causes hyperal-
gesia within 1 or 3 h, respectively, suggesting that NGF leads
to a relatively rapid sensitization of cutaneous nociceptors.
These rapid effects in the rat are thought to be mediated
primarily through NGF binding with TrkA expressed on
mast cells, causing degranulation and release of a variety of
allogenic mediators, such as histamine, prostaglandin E\textsubscript{2},
serotonin, hydrogen ions, and bradykinin, as well as addi-
tional NGF (fig. 1b), although the contribution of mast cells
is not as clear in humans. NGF can also be produced by
noninflammatory cells, such as keratinocytes\textsuperscript{65} and endo-
thelial cells,\textsuperscript{66} in addition to other inflammatory cells, such as
fibroblasts\textsuperscript{67} and T cells, in various \textit{in vitro} culture models.\textsuperscript{68}

The NGF-induced release of inflammatory mediators
from mast cells contributes to the sensitization of polymodal
nociceptors. In addition, NGF binds TrkA receptors
expressed on the peptidergic fiber terminal (fig. 1), leading to
sensitization of primary afferent nociceptors to thermal and
chemical stimuli \textit{in vitro} and \textit{in vivo}.\textsuperscript{69,70} This NGF-TrkA
activation of intracellular signaling cascades in the primary
afferent neurons results in sensitization or increased expres-
sion of a number of receptors and channels at the membrane
surface, including transient receptor potential vanilloid 1
(TRPV1), acid-sensing ion channels 2 and 3, endothelin
receptors, bradykinin receptors, voltage-gated sodium, and
calcium channels, delayed rectifier potassium currents, and
putative mechanotransducers,\textsuperscript{59,71–73} that contribute to im-
mediate hypersensitivity after inflammation (fig. 1b).

An important mechanism seen within minutes to hours of
NGF-TrkA binding is the sensitization of the heat-sensitive ion
channel TRPV1\textsuperscript{65,74} expressed by small-diameter peptidergic
fibers. Acute sensitization of TRPV1 by NGF may involve di-
rect phosphorylation, at least partly because of TrkA-mediated activation of p38 mitogen-activated protein kinases or phosphoinositide-3 kinase and disinhibition after hydrolysis of phosphatidylinositol-4,5-bisphosphate. Ultimately, sensitization of TRPV1 decreases the temperature threshold of sensory neurons to noxious heat. However, this does not happen at the level of individual TRPV1 channels recorded in dissociated DRG cells; the inward current response to noxious heat in...
increases as TRPV1 channels are translocated from the interior of the cell to the plasma membrane, but the temperature threshold does not change. Thus, any change in temperature threshold of a thermal nociceptor caused by NGF-induced sensitization of TRPV1 receptors results from a greater depolarization that causes the fiber to reach firing threshold at a lower temperature.

**Retrograde Transport of NGF-TrkA Drives Transcriptional Changes in Nociceptors**

After the period of immediate hypersensitivity with NGF release after tissue injury, early transcriptional changes occur in the sensory signaling pathway. Because NGF principally signals via retrograde transport of the internalized NGF-TrkA complex, there is a delay (from hours to days) before some of NGF’s contribution to hypersensitivity is seen. After retrograde transport to the DRG, the signal from the NGF-TrkA complex can produce changes in sensory phenotype through the switching on (and off) of gene promoters (fig. 2), which leads to increased synthesis of peptides (e.g., SP, calcitonin gene-related peptide [CGRP], and BDNF), and of nociceptor-specific ion channels (NaV 1.8, CaV 3.2, 3.3) at the DRG. For example, exposure of TrkA-positive sensory neurons to NGF increases expression of the nociceptive acid-sensing ion channel 3 via control of the promoter region of its gene. NGF-induced altered gene expression can also lead to a change in phenotype, whereby a population of sensory neurons switches from nonpeptidergic to peptidergic and becomes more responsive to NGF. Peripheral and dorsal horn terminals of peptidergic fibers express increased levels of peptides (SP, CGRP, and BDNF) as a result of these proteins being packaged and transported in the retrograde and anterograde directions from the soma (fig. 2). Indeed, systemic administration of anti-NGF neutralizing antibodies prevents the inflammation-induced up-regulation of neuropeptides (SP, CGRP) and the increased expression of the immediate early gene c-Fos in dorsal horn neurons without modifying swelling and erythema. The peptides, SP and CGRP on subsequent stimulation of the peptidergic primary afferent neurons, may contribute to an exaggerated inflammatory response. In addition, SP has been reported to cause local expression of NGF in keratinocytes.

**NGF, BDNF, and Central Sensitization**

A delayed phase of the inflammatory response to NGF (7 h to 4 d after NGF-TrkA binding in rodents) involves an indirect effect of NGF on synaptic transmission between nociceptors and second-order cells in laminae I and II of the spinal cord via its effect on the release of peptides such as BDNF (fig. 2). Evidence from 1994 suggests a role for the glutamatergic N-methyl-D-aspartate channel because NGF-induced behavioral hypersensitivity was selectively blocked by the noncompetitive N-methyl-D-aspartate receptor antagonist MK-801. The N-methyl-D-aspartate receptor plays a fundamental role in the development of wind-up and central sensitization, mechanisms that are thought to contribute to the development of facilitated sensory signals after injury. One potential mechanism believed to contribute to the development of central sensitization in the dorsal horn is...
Fig. 3. Nerve growth factor (NGF) induces sprouting and neuroma formation by sensory and sympathetic nerve fibers in a model of skeletal pain. Confocal images of periosteum of bone were acquired from whole mount preparations, tiled and overlaid (to scale) on a three-dimensional microcomputed tomography rendering of a sham femur (A) or sarcoma + vehicle femur (B), respectively, using Amira® software (Visage Imaging, San Diego, CA). Note that the tumor-injected femur (B) has significant cortical bone deterioration and a pathologic reorganization of calcitonin gene-related peptide (CGRP) nerve fibers (in red) compared with the sham bone (A). The boxed areas in (A) and (B) correspond to the confocal images in (C) and (D), respectively. High-power confocal images of nondecalcified whole mount preparations of the femoral periosteum from sham + vehicle (C)

Mechanisms of Pain Reduction via NGF-TrkA Inhibition

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The NGF-dependent up-regulation of BDNF in peptidergic nociceptors. In addition, BDNF is transported not only in a retrograde direction to peripheral terminals, but also in an anterograde direction from the DRG to terminals in the dorsal horn (see fig. 2). Upon release, BDNF acts as a central modulator via postsynaptic TrkB, the cognate receptor for BDNF. BDNF-TrkB binding on second-order cells can activate intracellular protein kinases, which can lead to phosphorylation of glutamate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. This phosphorylation has been shown to contribute to central sensitization at the dorsal horn synapse, particularly in combination with up-regulated peptides (SP and CGRP) acting on postsynaptic receptors (fig. 2). BDNF up-regulation after peripheral inflammation is NGF dependent because up-regulation is inhibited with administration of anti-NGF antibody. Behavioral observations indicate that antagonism of central BDNF attenuates the second (delayed) phase of hyperalgesia induced by formalin and the thermal hyperalgesia induced by carrageenan in an NGF-dependent manner, demonstrating a role for BDNF in hypersensitivity and pain. Collectively, the data suggest that BDNF-dependent activation of TrkB signaling is required for the development of the central sensitization process that underlies the development of persistent heat and mechanical hypersensitivity in the setting of tissue inflammation or injury.

These preclinical data point to a fundamental difference between the role of NGF during growth and differentiation, and its role in the adult sensory system when NGF-TrkA becomes a major player in the modulation and sensitization of a significant population of nociceptors that are involved in driving chronic pain. As NGF plays a prominent role in acute nociception and in mechanisms behind chronic hypersensitivity, there is a clear scientific rationale for interrupting NGF-TrkA signaling as a target for pain relief therapeutics.

NGF-TrkA–induced Sprouting and Neuroma Formation

One intriguing but largely unexplored mechanism by which NGF may also generate and maintain hypersensitivity is by inducing aberrant sprouting and/or neuroma formation in response to tissue and/or nerve injury. In previous studies in a rat model of neuroma, an NGF-sequestering fusion protein reduced both neuroma formation and the spontaneous, ectopic discharge that is a defining characteristic of painful neuromas. Other evidence suggests that local administration of NGF to normal peripheral nerves can also induce nerve sprouting of peptidergic (TrkA-positive) nociceptors.

NGF activation of TrkA-positive fibers has also been demonstrated to induce a remarkable reorganization of sensory and sympathetic nerve fibers. In a mouse model of bone cancer, it was shown that when osteosarcoma cells induce a tumor within bone, there is a remarkable sprouting and formation of neuroma-like structures by TrkA-positive sensory and sympathetic nerve fibers in the periosteum (fig. 3). This sprouting appears to occur within a week of tumor and tumor-associated stromal cells releasing NGF (fig. 3). Within this 1-week interval, these sensory and sympathetic nerve fibers appear to grow more than 1 mm in length and achieve a density never observed in normal bone (fig. 3). Sustained administration of an anti-NGF sequestering therapy largely blocked the pathologic sprouting of sensory and sympathetic nerve fibers and the formation of neuroma-like structures and significantly inhibited the generation and maintenance of cancer pain in this model (fig. 3).

A major issue in interpreting this remarkable and pathologic nerve sprouting is the source of the NGF driving this growth. Recent studies using canine prostate cells injected into the mouse bone shed light on the possible source of NGF because the canine prostate cells do not express NGF. After the prostate cells were injected into bone, sclerotic bone lesions similar to that found in human prostate cancer patients were observed, and TrkA-positive sensory and sympathetic nerve fibers innervating the prostate tumor-bearing bone marrow underwent a remarkable and pathologic sprouting. These prostate cells did not express detectable levels of messenger RNA coding for NGF, so these studies suggest that the source of NGF is not the tumor cells but rather NGF released by tumor-associated stromal, inflammatory, and immune cells, which frequently account for 10–80% of the cells comprising the tumor mass. These data demonstrate that even in the adult bone marrow, NGF released by these inflammatory, immune, and stromal cells can induce a 10- to 70-fold increase in density of TrkA-positive sensory nerve fibers in the bone marrow. The phenotype of these newly sprouted nerve fibers may be quite different from nerve fibers that innervate the normal bone

Fig. 3. (Continued) or sarcoma + vehicle (D) mice showing CGRP-positive nerve fibers and green fluorescent protein (GFP)-positive sarcoma cancer cells (green). When GFP-positive tumor cells invade the periosteum, they induce ectopic sprouting of CGRP-positive sensory fibers (D, arrow) and the formation of neuroma-like structures. Administration of NGF sequestering therapy (10 mg/kg; intraperitoneal, given at d 6, 12, and 18 after cell injection) reduces sarcoma-induced nerve sprouting of CGRP-positive (E), 200-kd neurofilament (NF200)-positive (F), and tyrosine hydroxylase (TH)-positive (G) nerve fibers at d 20 after cancer cell injection. Nerve fiber density was determined by measuring the total length of nerve fibers per unit volume in the periosteum. *P < 0.05. Bars represent the mean ± SEM. Reproduced and modified from Mantyh WG, Jimenez-Andrade JM, Stake BJ, Bloom AP, Kaczmarska MJ, Taylor RN, Freeman KT, Ghilardi JR, Kuskowski MA, Mantyh PW: Blockade of nerve sprouting and neuroma formation markedly attenuates the development of late stage cancer pain. Neuroscience 2010; 171:588–98, with permission from Elsevier.
Anti-NGF attenuates fracture-induced pain in mice

TrkA inhibition attenuates fracture-induced pain in mice

Time spent guarding during a 2-min period (sec)

Days post fracture

A

B

Sham + vehicle
Fracture + vehicle
Fracture + anti-NGF

p< 0.05 vs. Fracture + vehicle

Sham + vehicle
Fracture + vehicle
Fracture + TrkA inhibitor

p< 0.05 vs. Fracture + vehicle

Fig. 4. Therapies that sequester nerve growth factor (NGF) or inhibit tropomyosin-related kinase A receptor (TrkA) demonstrate significant analgesic efficacy in mouse and a human model of nonmalignant skeletal pain. In a mouse model of bone fracture, pain-related behaviors (the time spent guarding of the fractured limb during a 2-min observation) were significantly reduced by anti-NGF therapy (10 mg/kg, intraperitoneal, administered at d 1, 6, and 11 after fracture) (A) and the pan-Trk antagonist ARRY-470 (30 mg/kg, oral, administered twice daily beginning on d 1 after fracture) (B). Note that anti-NGF therapy (A) and the pan-Trk inhibitor (B) both reduced nonmalignant fracture pain-related behaviors by approximately 50%. Anti-NGF therapy reduced walking pain in human patients with moderate to severe osteoarthritis pain (C). The patient’s assessments of knee pain while walking in response to therapy were obtained at baseline and at the indicated times with the use of a visual analog scale that ranged from 0 to 100. In the case of knee pain, a decrease in the score indicates improvement (i.e., less pain). Changes are reported as least-squares means ± SE. *P < 0.001 for the comparisons of all doses of anti-NGF (tanezumab) with placebo in the assessment of knee pain, except for the comparison of tanezumab, 10 μg per kilogram of body weight, with placebo in the patient’s global assessment, for which P = 0.001. Reproduced with permission from Koewler NJ, Freeman KT, Buus RJ, Herrera MB, Jimenez-Andrade JM, Ghilardi JR, Peters CM, Sullivan LJ, Kuskowski MA, Lewis JL, Mantyh PW: Effects of a monoclonal antibody raised against nerve growth factor on skeletal pain and bone healing after fracture of the C57BL/6J mouse femur. J Bone Miner Res 2007; 22:1732–42, with permission from John Wiley & Sons.
and, as such, these newly sprouted nerve fibers may provide an anatomical substrate that drives skeletal pain. In support of this hypothesis, preventive treatment with an antibody that sequesters NGF, administered when prostate tumor-induced pain and bone remodeling are first observed, blocks the ectopic sprouting and significantly attenuates the development and severity of cancer pain.104

Sprouting of presumptive TrkA-positive nerve fibers has also been observed in nonmalignant skeletal pain states in human and animals. For example, studies have reported that in humans with chronic discogenic pain, there is growth of CGRP-positive nerve fibers into normally aneural and avascular areas of the intervertebral disc.107 Other studies have demonstrated significant sprouting of CGRP-positive nerve fibers after bone fracture in rat and in the arthritic joints of humans and animals.108–111 These reports suggest that after injury or disease of the skeleton, significant sprouting of TrkA-positive nerve fibers can occur, and it appears that endogenous stromal, inflammatory, and immune cells are a major source of NGF.68,105,106

These data on the ectopic sprouting of TrkA-positive sensory and sympathetic nerve fibers indicate how preemptive treatment with therapies that block NGF activation of TrkA may reduce the attendant pain but also block the pathologic remodeling of sensory and sympathetic nerve fibers that is a major driver of chronic hypersensitivity. This might be relevant in situations in which one can predict that tissue/nerve injury is about to occur, such as before amputation or orthopedic surgery, or when disease progression is highly likely, such as in osteoarthritis, pancreatic cancer, or tumor metastasis to bone.

NGF-TrkA Interactions and Chronic Pain: Preclinical Evidence

Anti-NGF Reduces Pain in Animal Models

A number of strategies have been developed to investigate the role of endogenous NGF in chronic pain. Most commonly, anti-NGF antibodies or a TrkA-IgG fusion protein to sequester NGF have been developed to block the biologic activity of NGF. Alternatively, it is possible to prevent NGF binding and activation of TrkA, for example with anti-TrkA antibody or a small molecular inhibitor of TrkA, although NGF activity via p75 will remain intact. These approaches have provided additional evidence for the role of NGF in acute and chronic hypersensitivity in adult animals after inflammatory injury.

The systemic administration of anti-NGF antibody has been shown to prevent the acute thermal45,60 and mechanical hyperalgesia induced by complete Freund’s adjuvant,60 whereas administration of a TrkA-IgG fusion protein minimized behavioral symptoms of hyperalgesia induced by carrageenan112,113 or ultraviolet B radiation.62 In addition, although not considered in detail here, in models of visceral inflammatory pain, hyperalgesia is markedly reduced by pretreatment with an NGF-neutralizing antibody or TrkA-IgG fusion molecule, for example in acetic acid-induced gastric inflammation,114 trinitrobenzene sulfonic acid-induced colonic hypersensitivity,115 and turpentine- or acrolein-induced cystitis.116,117 Furthermore, in a model of colitis, trinitrobenzene sulfonic acid-induced colonic hypersensitivity was also reversed by administering an anti-NGF antibody.115

Antibodies to NGF reversed the established hyperalgesia in a rodent model of autoimmune arthritis,61 suggesting that NGF is involved in prolonged hyperalgesia. In addition, the NGF-neutralizing antibody was at least as effective as indomethacin,61 used clinically for relieving arthritis pain. A role for NGF in maintenance of hypersensitivity in chronic injury has also been demonstrated using a model of bone cancer103,118 and a model of closed femur fracture119,120 (fig. 4).

Indeed, anti-NGF produces a profound reduction in ongoing and movement-evoked bone cancer pain-related behaviors that is greater than that achieved with acute administration of morphine.103,118

Early preclinical experiments modeling long-term NGF deprivation by active immunization of adult animals to autoreproduce antibodies against NGF demonstrated a reduction in the number of peripheral DRG fibers compared with untreated controls.121,122 This reduction was selective for unmyelinated C-fibers and was associated with diminished responsiveness to nociceptive stimuli.123 However, in later studies that used passive immunization, in which antibodies raised against NGF or TrkA were injected into mature animals, normal nociceptive function remained intact with minimal loss of functional sympathetic or sensory neurons.118,119 Such anti-NGF antibody treatment reduces pain caused by fracture or tumor growth in bone by about 50%,118–120 despite no reduction in the number of peripheral sensory or sympathetic nerve fibers innervating the skin or bone.102,118

One unique aspect of the sensory innervation of bone and joint, which may partially explain why anti-NGF therapy is effective in relieving malignant and nonmalignant skeletal pain, is that more than 50% of nerve fibers innervating bone are CGRP-positive fibers,52 nearly all of which coexpress TrkA (fig. 5).123 Accordingly, few unmyelinated nonpeptidergic (IB4-positive/RET-positive) nerve fibers are present
In bone, so therapies that target NGF or TrkA may be particularly efficacious in relieving bone pain where the tissues are innervated by nociceptors that express TrkA and respond to NGF.

Importantly, preventing NGF-TrkA signaling does not appear to compromise normal physiologic responses to injury, which are critical for effective healing. For example, NGF blockade does not affect the normal inflammatory response (erythema, heat, and swelling). In addition, at least cursory examination of anti-NGF therapy reveals no inhibition of NGF-TrkA signaling, is effective in reducing hypersensitivity in animal models. Importantly, the studies discussed suggest that, at least at the time points examined, this approach does not obviously compromise normal nociceptor function or cause the loss of sympathetic or sensory nerve fiber innervation of the skin or bone.

**Fig. 5.** There are differences in the percentages of tropomyosin-related kinase A receptor (TrkA)-positive sensory nerve fibers that innervate the bone versus skin. The skin is innervated by thickly myelinated A-β fibers (TrkA-negative), thinly myelinated Aδ fibers (both TrkA-negative and TrkA-positive), unmyelinated peptide-rich C fibers (TrkA-positive), and unmyelinated peptide-poor C-fibers (TrkA-negative). In contrast, the bone appears to be predominantly innervated by thinly myelinated Aδ fibers (mostly TrkA-positive) and peptide-rich C-fibers (also mostly TrkA-positive). The percentages and types of sensory nerve fibers innervating the skin and bone were estimated from previous studies. Thus overall more than 80% of all sensory nerve fibers that innervate the bone are TrkA-positive, whereas only 30% of the sensory nerve fibers that innervate skin are TrkA-negative, which might help explain why blocking nerve growth factor or TrkA is highly efficacious in attenuating skeletal pain.

Reproduced with modifications from Castañeda-Corral G, Jimenez-Andrade JM, Bloom AP, Taylor RN, Mantyh WG, Kaczmarska MJ, Ghilardi JR, Mantyh PW. The majority of myelinated and unmyelinated sensory nerve fibers that innervate bone express the tropomyosin receptor kinase A. Neuroscience 2011; 178:196–207, with permission from Elsevier Ltd.

**NGF-TrkA Interactions and Pain: Human Studies**

In humans, as in animal models, subcutaneous NGF evokes long-lasting mechanical hyperalgesia. Furthermore, NGF is locally up-regulated in humans presenting with chronic pain, such as arthritis, migraine/headache, fibromyalgia, or peripheral nerve injury. These observations suggest that in humans, as in preclinical animal models, the ongoing production of NGF may be involved in chronic pain and changes in sensitization. Indeed, there are at least three major pharmacologic strategies under development that target NGF-TrkA signaling for the treatment of chronic pain and that have produced effective reduction in hypersensitivity in preclinical models. These are sequestration of NGF or inhibiting its binding to TrkA, antagonizing TrkA so as to block NGF from binding to TrkA, and blocking TrkA kinase activity.

Among the first such molecules to be investigated preclinically were a TrkA-IgG fusion protein, MNAC13, and PD90780, which act by inhibiting the binding of NGF to TrkA and ALE0540, which appears to act by modulating the interaction of NGF with p75 and indirectly affecting TrkA activation. Although several of these molecules showed efficacy in reducing nociceptive behaviors, they were not advanced into clinical trials because of specificity or immunologic response issues. For instance, ALE0540 does not appear to have sufficient selectivity when compared with other tested receptors in vitro, MNAC13 is a mouse monoclonal antibody unsuitable for use in humans, and TrkA-IgG contains the extracellular domain of a normal human receptor (TrkA) and thus is likely to have significant consequences if immunogenicity develops. This potentially would be similar to the problems seen in rare patients treated with recombinant analogs of erythropoietin when they became immune to their endogenous erythropoietin. In contrast, a number of humanized anti-NGF monoclonal antibodies—RN624 (tanezumab), JNJ-42160443, REGN475, PG110, α-D11, AMG-403, which exert their analgesic effect by sequestering endogenous NGF—are being investigated in clinical trials in patients with various types of chronic noncancer pain. The outcomes of these clinical trials will provide key insights.
information on the efficacy of anti-NGF antibody therapy for the relief of pain in patients with different forms of chronic pain. Importantly, in studies published to date and in line with preclinical studies, anti-NGF therapy appears to be antihyperalgesic (i.e., normalizing a decreased nociceptive threshold) as opposed to analgesic (i.e., increasing normal and sensitized nociceptive threshold). Long-term studies are needed to enable a comparison of the safety profile of anti-NGF antibody therapy with those of currently used analgesic agents for chronic non-cancer pain, for which adverse side effects include gastrointestinal problems and potential cardiovascular risks. In addition, the safety profile of anti-NGF therapies must be investigated in a range of patients with different types of chronic pain.

**The Potential for NGF-TrkA Therapeutics**

Ultimately, the utility of NGF antagonism for pain relief in humans will depend on the contribution of the various NGF signaling pathways to the specific chronic pain condition. It is likely that not all types of pain are effectively reversed by antagonizing NGF-TrkA signaling. This therapeutic approach clearly relies on NGF being an important driver of the increased pain sensitivity; if other factors are responsible for driving the hyperalgesic state, inhibition of NGF may not be effective. For example, target-derived NGF is lost in conditions such as diabetes in which peripheral fibers suffer damage, a condition often accompanied by pain. Here, NGF might be expected to improve regeneration, thereby reducing pain. However, this approach was abandoned in patients with diabetic peripheral neuropathy because of dose-limiting painful side effects.

Nerve growth factor may be primarily involved in the initiation of changes that lead to chronic pain and may not itself have a prominent role in maintenance of hypersensitivity. Thus, the stage at which NGF is important in the development of ongoing hypersensitivity needs to be defined. Moreover, the extent to which signaling pathways are interlinked may limit their use clinically and in the interpretation of preclinical results. For example, anti-TrkA antibodies should suppress TrkA signaling, but they may also affect p75 signaling because there is speculation that the two pathways interact. In addition, specific nociceptor innervation of each tissue may influence the efficacy of NGF-TrkA–blocking strategies. Preclinical investigators who have focused on skeletal pain have proposed that anti-NGF treatment may be particularly effective in pain that originates in bone because more than 50% of the nociceptors that innervate bone are responsive to NGF.

To optimize the therapeutic potential of NGF inhibitors, additional research is needed to establish which types of human chronic pain are driven by and, more importantly, maintained by NGF. It is also important to understand when in the disease process NGF antagonism is most effective. For example, the pain that immediately follows bone fracture (from seconds to minutes later) is not inhibited by treatment with anti-NGF antibody in preclinical studies, whereas 24 h after fracture, anti-NGF therapy reduced bone fracture pain by more than 50% (fig. 4). This may indicate that initial nociceptive signals are driven by activation of, for example, mechanotransducers independent of NGF, whereas secondary nociception that occurs hours to days after fracture may be increased by the release of NGF, contributing to activation and sensitization of nociceptors. Additional study is needed to evaluate the putative effects of anti-NGF on other disease processes, such as weight loss in autoimmune arthritis and bone loss in the chronic pain condition known as complex regional pain syndrome I.

In addition to defining the analgesic efficacy of blocking the NGF-TrkA axis, key safety issues that need to be addressed with any therapy targeting NGF or TrkA include effects on normal autonomic and sensory neuron structure and function; physiologic responses to injury, wound healing, and endocrine function; ability to cross the placental or blood–brain barriers in the normal or injured state; and thus any influence on central nervous system neurons, such as the basal forebrain cholinergic neurons that are sensitive to NGF. In addition, given that bone pain may be a major target for NGF-TrkA therapies, understanding how these therapies affect individuals with advanced bone degeneration will be critical. Indeed, recent human clinical trials in elderly humans with osteoarthritis have been halted because of the need for earlier-than-expected joint replacement in a small subset of patients. Whether this earlier-than-expected joint replacement in patients being treated with anti-NGF is attributable to greater use of the diseased joint or unforeseen adverse events on the biomechanical properties of bone itself remains a critical but unanswered question. These data emphasize the need to understand clearly not only the analgesic efficacy of TrkA-NGF blocking therapies and any unexpected effects but also how patients with chronic pain change their behavior and use of the injured/degenerating tissue after administration of a therapy that provides significant pain relief without sedation.

**Conclusion**

This review provides an overview of the mechanisms by which NGF drives acute and chronic pain in the adult and outlines how NGF has a distinct role in the adult compared with the developing nervous system. To date, therapies that target NGF-TrkA signaling have shown significant analgesic efficacy in animals and humans in several difficult-to-treat chronic pain states. In choosing which chronic pain states to target with NGF-TrkA therapies, a key issue to consider is the fraction of NGF-responsive (TrkA-positive) nociceptors that innervate the tissue from which the pain is arising because this innervation varies considerably from tissue to tissue. If successful, therapies that target NGF-TrkA signaling represent a new class of analgesic therapy that has the potential to change the therapeutic landscape of how we treat several types of chronic pain.
38. Johnson EM Jr, Osborne PA, Taniuchi M: Destruction of sympathetic and sensory neurons in the developing rat by a monoclonal antibody against the nerve growth factor (NGF) receptor. Brain Res 1989; 478:166–70
60. Bishop T, Hewson DW, Yip PK, Fahey MS, Dawbarn D, Young AR, McMahon SB: Characterisation of ultraviolet-B-induced inflammation as a model of hyperalgesia in the rat. Pain 2007; 131:70–82
64. Foster PA, Costa SK, Poston R, Houltr J, Brain SD: Endothelial cells play an essential role in the thermal hyperalgesia induced by nerve growth factor. FASEB J 2005; 17:1703–15
71. Lewin GR, Ritter AM, Mendell LM: Nerve growth factor...


73. Zhang YH, Vasko MR, Nicola GD: Ceramide, a putative second messenger for nerve growth factor, modulates the TTX-resistant Na\textsuperscript{+} current and delayed rectifier K\textsuperscript{+} current in rat sensory neurons. J Physiol 2002; 544:385–402


104. Jimenez-Andrade JM, Bloom AP, Stake JI, Mantyh WG, Taylor RN, Freeman KT, Ghilardi JR, Kuskowski MA, Mantyh PW: Pathological sprouting of adult nociceptors in chronic...
130. Iannone F, De Bari C, Dell’Accio F, Covelli M, Patella V, Lo Bianco G, Lapadula G: Increased expression of nerve growth factor (NGF) and high affinity NGF receptor (p140 TrkA) in human osteoarthritic chondrocytes. Rheumatology (Oxford) 2002; 41:413–8
gests altered NGF-p75NTR interactions in the presence of TrkA.


144. Mendell LM: Does NGF binding to p75 and TrkA receptors activate independent signalling pathways to sensitize nociceptors? J Physiol 2002; 544:3–5


149. Ambalavanar R, Moritani M, Dessem D: Trigeminal P2X3 receptor expression differs from dorsal root ganglion and is modulated by deep tissue inflammation. Pain 2005; 117:280–91
