Manzanita
PHARMACEUTICALS

An historical review of selected functions of exogenous Nerve Growth Factor: selective binding, endocytosis, and axonal transport

Kahl S.B.,* Burton L.E., ** Hill G.C.,* and McKee, C.A.***

* PhD, Chief Scientific Officer, Manzanita Pharmaceuticals, Inc., Woodside, CA, US; ** PhD, consultant to Manzanita Pharmaceuticals, Inc., LGB Consulting, San Mateo, CA, US; *** PhD, consultant to Manzanita Pharmaceuticals, Inc.; Director, Radiopharm Development and Translation, SpectronRx, Indianapolis, IN, US; * MBA, Manzanita Pharmaceuticals, Inc., Woodside, CA, US.

1 EXECUTIVE SUMMARY

Introduction. Nerve Growth Factor (NGF) was discovered by Rita Levi-Montalcini MD PhD in 1952 [1] (Hamburger 1949 [2]; Cohen 1954 [3]; Levi-Montalcini 1976 [4]; Aloe 2004, 2012 [5, 6]). Nerve Growth Factor has been considered as a therapeutic agent for multiple, mostly neurodegenerative conditions (Rocco 2018 [7]). This review does not consider NGF as a potential, directly acting therapeutic agent. Rather, the scientific case is considered for NGF as a delivery facilitating moiety, to target the intraneuronal environment of peripheral nerves.

Historical review: methodology. This review considered the published literature as to exogenous administration of NGF selective binding, internalization (endocytosis or phagocytosis), and axonal transport. This is not a systematic, but an historical narrative that identifies references from key papers as the main process. The review was conducted independently of the review paper, “Receptor binding, internalization, and retrograde transport of neurotrophic factors” (Neet and Campenot 2001 [8]).

One outcome of this review was noting that most of the basic research into the selective binding, internalization, and axonal transport of NGF appears to have been completed by 2000. Since then, research has focused on various defects involving these processes in disease.

Product in development: the Nerve Growth Factor-fluorescent dye conjugate. This review examines the published literature of selected pharmacokinetics (PK) of Nerve Growth Factor (NGF), namely the emphasis on the mechanism and timing of binding to its receptors, whether the endocytosis of conjugates was possible, and the velocity of absorption, or retrograde axonal transport. Separately, we compared the published literature to results to date of our fluorescent dye-NGF conjugate, 800-rhNGF.

We are developing 800-rhNGF, a conjugate in which a known fluorescent dye in the 800 nanometer (nm) region is attached directly to amino acids located on the surface of recombinant human Nerve Growth Factor, NGF (800-rhNGF). Proprietary synthetic protocols leave both the dye free to fluoresce and NGF free to bind to its high and low affinity receptors, both of which are known. The first indication for 800-rhNGF is as a surgical guidance tool, a nerve imaging agent to be used in radical prostatectomies, in which localized, mostly early-stage prostate cancer is resected surgically.

After this review of the NGF literature, two separate comparisons evaluated whether the published NGF literature supported (i) the results of 800-rhNGF observed in non-GLP (GLP, Good Laboratory Practice) in rat studies to date (n=46) - yes; and (ii) the criteria for clinical workflow, as defined to us unanimously in detailed due diligence questions to urologic cancer surgeons – yes. Not reviewed are the characteristics of an 800 NIR dye which would also meet clinical criteria. Nonclinical results of 800-rhNGF will be published separately. This review focuses solely on what is known of the pharmacokinetics and science of NGF.

- **Binding mechanism of NGF partly explains selectivity.** It is definitive that (i) NGF binds with high affinity for TrkA receptors; (ii) NGF also binds to the low affinity, “pan-neurotrophin” p75 receptor; (iii) binding to TrkA occurs within 3-6 min, to p75 within seconds (in PC12 cells, Godfrey and Shooter 1986; Senger and Campenot 1997 [9, 10]); (iv) TrkA and relatively more p75 receptors (percentage unknown) are expressed at the distal ends of nerves (Godfrey and Shooter 1986 [9]); and (v) TrkA is genetically encoded, and is highly homologous in all mammals, including humans ([11-13]).

For example, in an oncological resection of the prostate, the peri-prostastic space ("bed" of the initial surgical incision) enables access to TrkA and p75 receptors newly exposed after surgical incision. The other part of selectivity is that 800-rhNGF will 'pool' in the surgical space created by the incision. The relative benefits of localized vis-à-vis systemic administration are not reviewed here.

Table 1. Criteria for clinical utility for a nerve imaging agent to aid radical prostatectomies

Application	At beginning of procedure, intra-operatively, interstitially (topically) to the peri-prostatic space (bed of initial surgical incision). No need to target any particular anatomical feature;
Wash	Wash with saline three times (3X) after 15-30 min;
Durable	Can be imaged at end of procedure, ~ 2h;
Safe	Degrades safely, so patient can be sutured up at the end of ~ 2h surgery;
Clinical goal	Does not interfere with primary goal, which is cancer control (does not tell surgeons what to do); and
Benefits	Intra-operative, intrastitial (topical, peri-prostatic) application should reduce systemic dose. Localized application of 800-rhNGF is likely to reduce patient in-surgery time, and reduce hospital cost if less time is spent in surgery and/or in hospital, if a day in hospital is spent if a nerve imaging agent is injected intravenously (IV).

Not all nonclinical experiments distinguished carefully between whether the pro or mature form of NGF was used. It is critically important to understand this selection, since only the mature form binds to TrkA receptors (Luberg 2015; Fahnestock 2001; Ioannou and Fahnestock 2017; Shekari and Fahnestock 2019 [14-17]). For example, the 800-rhNGF nerve imaging agent under development uses only the mature form of NGF. As discussed further in Appendix D, "oncogene" describes the pro form of NGF, which is produced endogenously in adult mammals [18], but proNGF does not bind TrkA.

Endocytosis. The term endocytosis correctly describes endocytosis ("internalization") of all proteins. What is reviewed here is the endocytosis of NGF-TrkA-(p75). It is known that the NGF-TrkA complex is moved intraneuronally, specifically on a 'surface-bound' path, on the outside of microtubules (Peters 1968, 1991 [19, 20]; Rodriquez Echandia 1968 [21]; Burton P.R. 1984 [22]; Ure and Campenot 1997 [23]; Garvalov 2006 [24]).

Not reviewed are the specific mechanisms or the destination sites of transport of anterograde transport. What is not definitive is what primary and secondary signals are sent after NGF-receptor binding, and by what, to initiate endocytosis and then fast and slow retrograde axonal transport. Key findings from this review are:

(i) NGF is endocytosed with receptors TrkA and/or p75;

(ii) The NGF-receptor complex is probably transported in clathrin-coated vesicles (Howe 2001 [25]; Brown 2013 [26]);

(iii) NGF has been previously modified to involve even larger complexes than by comparison, the 800-rhNGF conjugate under development (mature form NGF kDA 26.3 is bound to fluorescent dye, MW 1015); and

(iv) Once endocytosed, NGF-TrkA is loaded onto the retrograde axonal transport system with relatively high - 85% - efficiency (Ure and Campenot 1997 [23]).

Axonal transport. It is definitive that:

(i) Axonal transport includes NGF and other proteins;

(ii) There are fast (Brady 1984, 1993 [27, 28]; Brady 1985b [29]; Treanor 1995 [30]; Senger and Campenot 1997 [10]; Butowt and von Bartheld 2009 [31]) and slow components of axonal transport (Ure and Campenot 1997 [23]; Senger and Campenot 1997 [10]). The half-life of slow, retrogradely transported NGF *in vitro* is estimated at 6h (Ure and Campenot 1997 [23]);

(iii) Slow and fast axonal transport begins retrogradely (from the periphery to the neuronal cell body) (Hendry 1974a [32]; Brimijoin and Helland 1976 [33]; Allen 1982 [34]; Brady 1982 [35]; Stenoien and Brady 1999 [36]; Butowt and von Bartheld 2009 [31]);

(iv) In the neuronal cell body, the NGF-receptor complex is degraded into non-toxic components primarily by proteolysis - by nucleases, proteases, esterases, glycosidases, lipases, phosphatases and sulfatases

(Avers 1982 [37]; Sheeler 1983 [38]; Parton and Dotti 1993 [39]; Hosang and Shooter 1986 [40]; Vissavajjhala 1992 [41]; Neet and Campenot 2001 [8]; Boutilier 2008 [42]; Frampton 2012 [43]); and

(v) Degraded, non-toxic NGF-receptor fragments are moved anterogradely ("orthograde" or anterograde axonal transport), back to the periphery, channeling various products into various neuronal channels (Sec 3.3 Stenoien and Brady 1999 [36]; Butowt and von Bartheld 2009 [31]).

Future publications of nonclinical results of 800-rhNGF. Nonclinical results of 800-rhNGF will be published in the future. Significantly, since the expression of Trk receptors is highly homologous in all mammals, the non-GLP results to date in rat are clinically predictive. After NGF binds to TrkA and/or p75 receptors, absorption of the NGF-receptor complex continues after wash, when the NGF-TrkA complex is loaded onto the retrograde axonal transport system. 'What the surgeon sees' in the display of an imaging system is the 800 dye - not indocyanine green (ICG) (Vahrmeijer 2013 [44]).

Ninety-nine per cent of the installed base of imaging systems are designed to detect a dye that fluoresces in the 800 nm region: indocyanine green (ICG). The ability to visualize the 800 dye in 800-rhNGF has been confirmed to date not only in all rat studies (total n=103), but also in two canine studies (n=2) that evaluated two different marketed imaging systems. Both those imaging systems were designed to detect ICG, and approved for marketing. This review does not discuss imaging systems. Future publication of nonclinical results of 800-rhNGF will include:

(i) Dose range-finding studies (completed in rat, 1.0 mg/ml for Dye-Adduct-Ratio DAR2);

(ii) Signal-to-Background Ratio (SBR) calculations, from nerve-to-muscle (not nerve-in-adipose tissue) measurements. As expected, SBR results reflect 'steady state' and the larger cargo size of the DAR2 variant: the SBR DAR2 was only ~ 25% higher than DAR1, even though number of molecules doubled in the DAR2 variant from the DAR1 variant. In addition to the bioconjugation parameters, key determinants were also the "brightness" of 800-rhNGF and sensitivity of the imaging systems;

(iii) Histology, using co-localization, TrkA was confirmed as a binding mechanism for 800-rhNGF. Neither the binding of NGF to p75 nor the TrkA-p75 complex were assessed histologically; and

(iv) Canine studies, within the terms of the Non-Disclosure Agreements (NDAs) signed with three firms to date. A fourth has expressed interest.

Contents of this Review

- In Sections 1 – 3 below, we review the selective binding, endocytosis, and axonal transport of NGF.
- Section 4 summarizes key findings from this review.

March 2024

1 STEP ONE: SELECTIVE BINDING OF NERVE GROWTH FACTOR TO TROPOMYOSIN KINASE A RECEPTOR IS GENETICALLY ENCODED

Table 1. Criteria for clinical utility for a nerve imaging agent to aid radical prostatectomies

Application	At beginning of procedure, intra-operatively, interstitially (topically) to the peri-prostatic space (bed of initial surgical incision). No need to target any particular anatomical feature;
Wash	Wash with saline three times (3X) after 15-30 min;
Durable	Can be imaged at end of procedure, ~ 2h;
Safe	Degrades safely, so patient can be sutured up at the end of ~ 2h surgery;
Clinical goal	Does not interfere with primary goal, which is cancer control (does not tell surgeons what to do); and
Benefits	Intra-operative, intrastitial (topical, peri-prostatic) application should reduce systemic dose. Localized application of 800-rhNGF is likely to reduce patient in-surgery time, and reduce hospital cost if less time is spent in surgery and/or in hospital, if a day in hospital is spent if a nerve imaging agent is injected intravenously (IV).

1.1 Key points

The processes of selective binding, endocytosis, and axonal transport have been reported for many proteins. This review considers these processes only as they relate to Nerve Growth Factor (NGF). Although binding, endocytosis, and axonal transport in reality are a continuous process, defining them separately as distinct processes is arbitrary, but convenient for discussion.

There is a body of evidence that NGF binds to both the high affinity tropomyosin kinase A (TrkA) and the low affinity ‘pan-neurotrophin’ p75 receptors. The “NGF binds to TrkA and p75” idea is deliberately simplistic because TrkA can be tested histologically. Not discussed are the isoforms of TrkA (Luberg 2015 [14]) or possibly non-genetic factors such as TrkA receptor co-factors and/or co-receptors (Trouvilliez 2023 [45]).

Complicating the interpretation and/or reliability of published reports is whether the NGF uses the pro or the mature form of NGF: only the mature form binds to TrkA (Kliemannel 2007 [18]). The neurotrophin BDNF and its high affinity receptor TrkB have functions like the NGF-TrkA receptor. Like NGF, BDNF occurs in both the pro and mature form of protein, and the TrkB receptor may promote apoptosis or growth (Mandel 2009 [46]).

This review discusses first TrkA, then p75, before considering the evidence of their co-occurrence. It is definitive that -

(i) NGF binds with high affinity for tropomyosin kinase A receptors, or TrkA (not tyrosine kinase);

(ii) TrkA is genetically encoded in all mammals, including in humans;

(iii) NGF also binds to the low affinity, pan-neurotrophin p75 receptor;

(iv) Binding to TrkA takes place within 3-6 min; binding to p75 may occur within seconds;

(v) TrkA and p75 receptors are expressed only at the distal ends of sensory nerves; and

(vi) When TrkA and/or p75 are bound, it is known that phosphorylation occurs almost immediately, but then signaling does also. However, the complex process of signaling has not been elucidated.

What is not definitive is the sequence of when and under what circumstances TrkA and p75 are complexed, nor is it known what signals are sent after their binding and complexation. In the ‘steady state’ that characterizes slow retrograde axonal transport (Ure and Campenot 1997 [23]), the NGF-TrkA complex – where TrkA receptors are saturated - is transported to and degraded in the neuronal cell body, then recycled proteolytic materials are transported anterogradely (Stenoien and Brady 1999 [36]; Butowt and von Bartheld 2009 [31]). The concept of ‘steady state’ may be further modified by cargo size (Roy 2014 [47]).

In nonclinical results, the NGF-fluorescent dye conjugate 800-rhNGF was designed to leave the TrkA binding sites of NGF unimpeded after bioconjugation. Selective binding occurred in 100% of experiments, regardless of dose and/or regardless of Dye-Adduct-Ratio (DAR, number of dye molecules per NGF) (total studies n=103). We attributed this effect due to the robustness and ubiquity of the TrkA binding mechanism. ‘What the surgeon sees’ in ICG-enabled equipment is the dye, not NGF. Although the p75 receptor is not specific to sensory neurons, when 800-rhNGF is applied to a compartment, *e.g.,* peri-prostatic space in radical

prostatectomies, it should localize mainly to sensory nerves via TrkA and/or p75 receptor binding, then endocytosis, and retrograde axonal transport for the duration of the surgical procedure.

It is its high affinity for TrkA receptors that enables the selectivity of 800-rhNGF for sensory neurons only. Although some consider the sensory-nerve-only parameter a restriction, our view is that any restriction is outweighed by the benefits of NGF selectivity.

1.2 Seventy years since the discovery of Nerve Growth Factor

Nerve Growth Factor (NGF) was discovered by Rita Levi-Montalcini MD PhD in 1952 [1] (Hamburger 1951 [2]; Cohen 1954 [3]; Levi-Montalcini 1976 [4]; Aloe 2004, 2012 [5, 6]). Observing initially that NGF acted on both the "survival and differentiation of selected populations of peripheral neurons," NGF was considered as a therapeutic agent for multiple, mainly neurodegenerative conditions (Rocco 2018 [7]).

Broadly, neurotrophins including NGF are mainly produced endogenously by glial cells (Heumann 1987a,b [48, 49]; Acheson 1991 [50]; Yoshida and Gage 1992 [51]), peripheral neurons in the peripheral nervous system (PNS) (Ernfors 1992 [52]; Schecterson and Bothwell 1992 [53]; Acheson 1995 [54]), and in the brain and spinal cord, in the central nervous system (CNS) (Richardson 1986 [55]). Nerve Growth Factor is produced during development and maturity, and with TrkA, endogenous NGF may designate survival or apoptosis (Yan 2002 [56]), or growth or maintenance.

Although the amount of endogenous NGF produced varies depending on the tissue of origin (Alastra 2021 [57]), it is practically ubiquitous: " …. Endogenous NGF is found in "every peripheral tissue/organ that is innervated by sensory afferents and/or sympathetic efferents" (Micera 2003 [58]; Lambiase 2004 [59]; Aloe 2012 [6]; Aloe 2015 [60]; Minnone 2017b [61]). In the PNS, NGF can be found in sensory, sympathetic and parasympathetic cells, cardiovascular cells, and autonomic neurons. Both the pro and the mature form occur endogenously (Kliemannel 2007 [18]; Fahnestock 2001 [15]; Hempstead 2014 [62]; Ioannou and Fahnestock 2017 [16]; Shekari and Fahnestock 2019 [63]).

The neurotrophin brain-derived neurotrophic factor (BDNF) and its high affinity receptor TrkB have similar functions as the NGF-TrkA receptor. This paper does not include an historical review of the selective binding of BDNF, endocytosis of the BDNF-receptor complex, and its axonal transport. Like NGF, BDNF occurs as both the pro and mature form, and where the TrkB receptor may promote apoptosis or growth (Mandel 2009 [46]).

1.3 Selective binding: significance

Like many biologic phenomena, selectivity does not mean exclusivity (*i.e.*, NGF does not bind exclusively to its high affinity receptor TrkA). The concept of selective binding of NGF to TrkA is significant because it can explain:

(i) How a nerve imaging agent such as 800-rhNGF constructed with NGF can localize to, and be absorbed mainly by at-risk nerves in the "new surface" exposed in the "bed" of a surgical incision; and

(ii) Why the two effects of binding and localization to the surgical space can be observed in multiple locations modeling other surgical incisions where sensory and/or motor neurons are involved.

Although this review focuses on NGF, it is known that BDNF binds to its high affinity receptor TrkB on, for example, the TrkB receptors expressed on Retinal Ganglion Cells (RGCs), a layer of cells in the posterior of the eye. Similar to NGF and its selective binding to TrkA, BDNF binds to TrkB and p75. It is believed that BDNF plays a role both in the neuroprotection of RGCs (Fudalej 2021 [64]).

The events required to transport BDNF after binding to its high affinity receptor TrkB are less well studied than for NGF and TrkA, but appear to be similar to those of NGF/TrkA (Kahl 2019 [65]). In cancer resections, for example, only sensory nerves are at risk in prostate cancer (cavernous nerves) and breast cancer reconstruction (axillary nerves). Not considered here is the conjugation of the same dye to rhBDNF (800-rhBDNF). Because they do not compete for the same receptors, NGF and BDNF may be combined for surgeries in which sensory-motor nerve groups are at risk. In principle the combination can address the remainder of all other cancer surgeries – sensory and motor nerve groups - without sacrificing selectivity.

1.4 Selective binding: endogenous and exogenous Nerve Growth Factor

It is well established that TrkA receptors are expressed mainly on the distal ends (nerve endings) of sensory (sympathetic) nerves (Toma 1997 [66]). Selective binding describes NGF's high affinity for, but not exclusive

binding to TrkA (Rodriguez-Tebar 1990, 1992 [67, 68]; Yancopolous 1990 [69]; Soppet 1991 [70]; Benedetti 1993 [71]; Windisch 1995 [72]; Belliveau 1997 [73]; Arévalo 2004 [74]).

All tropomyosin kinase (Trk) high affinity receptors – TrkA, TrkB, and TrkC - are encoded by Trk genes NTRK1, NTRK2, and NTRK3, respectively (Jiang 2021 [75]): for TrkA (Johnson 1978a, 1987; Martin-Zanca 1989 [76-78]; for TrkB (Klein 1989 [79], 1990a,b, 1991a,b [79-83]; Middlemas 1991 [84]; Squinto 1991 [85]); and for TrkC (Martin-Zanca 1986, 1989 [78, 86]; Lamballe 1991a [87]).

The cellular events required to bind, endocytose, and transport NGF retrogradely are known (Johnson 1978a; Hendry 1974a; Stoekel and Thoenen 1975; Goedert 1986; Palmatier 1984; Korsching and Thoenen 1988; Barde 1982; Cordon-Cardo 1991 [32, 76, 88-93]), and involve high affinity TrkA receptors (Johnson 1978a; Martin-Zanca 1989; Lamballe 1991a; Hempstead 1991; Barbacid 1991 [76, 78, 83, 87, 94, 95]) and low affinity, pan-neurotrophin p75 receptors (Roux and Barker 2002 [96]). In addition, guiding the synthesis of the 800-rhNGF conjugate so that NGF's receptor binding activity remains intact after synthetic manipulation, the amino acids - key external residues - that bind to NGF are also known (Wiesmann 1999 [97]; Ultsch 1999 [98]).

As noted by Palmatier 1984:

> "Many NGF effects are mediated by the binding of NGF to the receptor [TrkA] …. " (Loeb 1991 [99]; Loeb and Greene 1993 [100]; Ibáñez 1992 [101]; Senger and Campenot 1997 [10]). Whether physiological and/or enabled by signaling, selective binding of the NGF-TrkA complex also initiates the processes of internalization and retrograde axonal transport (Palmatier 1984 [90]).

Early studies *i.e.* Loeb 1991 and Loeb and Greene 1993 used a cell line, PC12 cells to confirm TrkA binding. Although this cell line reliably reports TrkA binding, cells do not recapitulate the neuroanatomy of axons and cannot be used to study retrograde transport. Table C.3 in Appendix C includes studies in PC12 cells that confirm receptor binding. We consider reports using PC12 as distinct from those that report axonal transport, *i.e.*, involve the neuroanatomy of microtubules.

Historically, two receptors for NGF – a high affinity ("slow") and low affinity ("faster") – were identified. The high affinity receptor was termed "gp140^{prototrk}," and was initially identified as a unique 143 kDA protein (Hosang and Shooter 1985 [40]). Then in 1991, it was the Shooter lab at Stanford University who elucidated the structure of the TrkA receptor as the larger of two receptors to which NGF binds (Meakin and Shooter a,b 1991 [102, 103]; Meakin 1992 [104]; Barker 1993, 1994 [105, 106]; Drinkwater 1993 [107]. Like TrkA, p75 is a transmembrane glycoprotein of 80-85 kDA (Chao 1986 [108]; Johnson 1986 [109]; Radeke 1987 [110]; Rodriguez-Tebar 1992 [68]).

Although not directly involved in that work in the Shooter group, Gene Burton, a co-author of this review, was in the Shooter lab at the time, but left Stanford in early 1982 for a post-doctoral position. Later, at Genentech, Burton wrote a book chapter on the manufacturing of neurotrophins (L.E. Burton [111]). Nerve Growth Factor was not patented. All manufacturing patents have since expired. The mature form of recombinant human NGF (rhNGF) may be easier to produce as a biosimilar because it is not glycosylated (L.E. Burton, personal communication).

Finally, the terminology for the tropomyosin kinase receptors that determine selective binding can be confused with terminology for their gene products – tyrosine kinase receptors (Jiang 2021 [75]). By 2017, the number of tyrosine kinase receptors that had been identified was at least 90. The tyrosine kinases are druggable ("tyrosine kinase inhibitors," TKIs). The first TKI developed as a therapeutic agent was approved in 2001: imatinib, or Gleevec™ (Espada 2017 [112]; Cohen 2021 [113]; Ip 1993a [114]).

1.5 Selective binding: bioassays recapitulate neuroanatomy, to model key processes

Remarkably, even in 1977, when the receptor now known as TrkA was not yet known, in a bioassay system that recapitulated neuroanatomy ("compartmented cultures"), Campenot observed that exogenous NGF was absorbed specifically at "peripheral nerve terminals" (Campenot 1977 [115]). Functionally, local application of NGF to distal ends (nerve terminals) of sympathetic neurons is sufficient to elicit a local growth response, including sprouting (Appendix H) *in vitro* (Campenot 1977 [116], Campenot 1982a [117], 1994 [118]) and *in vivo* (Miller 1994 [119].

The compartmented cultures bioassay separated the nerve terminals (distal ends) of Superior Cervical Ganglia (SCG) neonatal rat neurons physically and physiologically from nerve bodies (proximal ends). In its development, Campenot relied on his background in physics and evoked potentials, but also on previous results (Levi-Montalcini 1976 [4], 1980 [120]; Hendry 1974a [32]; Stöckel 1974 [121], Hendry 1975 [122];

Hendry 1974a, 1972, 1977, Hendry and Campbell 1976 [32, 123-125]; Hendry and Iversen 1973 [126]; Iversen 1975 [127]). Later, that bioassay system was adapted to add a middle chamber (Campenot 1982a [128]). Developing neurons have an absolute requirement for NGF, so neuronal survival indicates that the NGF component remains bioactive after synthetic manipulation (*i.e.,* retains its binding function) (Zareen 2009 [129]; Jackson and Tourtelotte 2014 [130]). Bioassays of SCGs are now in their next generation as microfluidic devices [131-134].

To biologically characterize 800-rhBDNF, an analogous bioassay exists for neuronal survival of BDNF and its high affinity receptor TrkB. That bioassay shows that BDNF retains its binding ability after synthetic manipulation (Kahl 2019 [65]). In a bioassay of BDNF function, binding to TrkB can be confirmed by neuronal survival in neonatal Retinal Ganglion Cells (RGCs) (Barres 1988, Winkler 2014 [135, 136]).

1.6 Selective binding: differential distribution of exogenously delivered neurotrophins

Experimentally, the selectivity of TrkA was tested in several studies in which various neurotrophins NGF, BDNF, and NT-3 for example, were introduced exogenously, via different routes. 'Differential distribution' describes the phenomenon whereby exogenous neurotrophins localize to neurons and neuronal locations expressing the Trk receptors that have high affinity for those neurotrophins.

In other words, selective binding has been observed for NGF and other neurotrophins, illustrating that Trk receptors – not just route of administration - guide selectivity. Differential distribution occurs regardless of route of administration due to the affinity of Trk receptors, *i.e.,* intracerebrally and intranasally (DiStefano 1992, Lindsay 1994, Anderson 1995 Mufson 1999, DiStefano 1994 [137-141]; Belliveau 1997 [73]; Curtis 1995 [142]).

1.7 Location of selective binding: TrkA and p75 at the distal ends

Key to the principle of 'local control' is the location of receptors at the distal ends only of sensory nerves. For example, in oncologic resections, previously buried nerve endings are exposed at a "new periphery" or "new surface." In 1997, Toma reviewed the NGF literature finding definitively that TrkA receptors are expressed on nerve terminals (distal ends) of NGF responsive neurons (Kim 1979 [143]; Carbonetto and Stach 1982 [144]; Rohrer and Barde 1982 [145]; Richardson 1986 [55]; Toma 1997 [66]).

A few reports provide evidence of the location of p75 receptors as adjacent to TrkA receptors, but the quantity of p75 receptors relative to TrkA (Godfrey and Shooter 1986 [9]; Miller 1994 [119]) remains unclear. Also unclear is where p75 receptors are located (Ross 1984 [146]; Sonnenfeld 1986 [147]; Zimmerman and Sutter 1983 [148]; Richardson 1986 [55]).

1.8 Binding of the pan-neurotrophin receptor p75

In addition to binding to the high affinity TrkA, all neurotrophins also bind to the low affinity, pan-neurotrophin p75 receptor, also referred to as NTRp75 (Chao 1994, 2003a [149, 150]; Barker 1994 [105]; Friedman 1999 [151]; Neet and Campenot 2001 [8]; Roux and Barker 2002, Roux 2001 [96, 152]). The p75 receptor 'enhances,' but does not compete with, the high affinity TrkA receptor (Hempstead 1991 [94]). The two receptors are trafficked differently (Curtis 1995 [142]; Pryor 2012 [153]). It is known that the pan-neurotrophin receptor p75 also undergoes fast retrograde transport (Hempstead 1991 [94]; Lalli and Schiavo 2002 [154]; Chao 2003a [150]; Bronfman 2003 [155]; review, Meeker and Williams 2015 [156]; Huang and Reichardt 2001 [157]).

1.9 Selective binding: clinical utility means being able to access TrkA and p75 receptors, and having sufficient time to bind

The clinical utility of an NGF-derived intra-operative product depends on having access to high- and probably low-affinity receptors exposed in the "new surface," and a sufficient distribution and density of those receptors on sensory nerve endings. In the handful of studies that we found that published TrkA "maps," we found it difficult to align the age of neurons in development with neurons at maturity (Korsching and Thoenen 1983b [158]; Richardson 1986 [55]); the type of nerves, *i.e.,* spinal nerves, Dorsal Root Ganglia (DRG) (Mu 1993 [159]; McMahon 1994 [160]; Muragaki 1995 [161]); or the species in which studies were done.

Further, the clinical utility of 800-rhNGF depends critically on the time required for NGF to bind to TrkA and p75. For TrkA, it has been reported that 3-6 min are needed for NGF to bind to receptors (Senger and Campenot 1997 [10]). The phosphorylation of TrkA begins within a minute of NGF binding to TrkA (Senger and

Campenot 1997 [10]; Campenot 2009b [162]). For the low affinity p75 receptor, binding can occur within seconds in PC12 cells (Godfrey and Shooter 1986 [9]).

1.10 The case for Nerve Growth Factor binding to both TrkA and p75

There is evidence that NGF binds to both TrkA and p75:

- Most ^{125}I-NGF binds within 3-6 min (Godfrey and Shooter 1986 within 5 min, in PC12 cells [9]; Senger and Campenot 1997 [10]; evidence for two receptors, Sutter 1979 [163]; Ure and Campenot 1997 [23]);
- In the earliest studies, there was evidence of the co-occurrence of two receptors, now known as TrkA and p75 (Hendry 1974b [164]; Stöckel 1975a [165]). However, the circumstances of co-occurrence have not been faithfully replicated due to their different roles in development and maturity, temperature, age of neurons, nerve group). Co-occurrence of receptors also seems to affect the duration of their effect: " …in the sensory neuron the selectivity of uptake and transport seems to persist over the whole life span whereas the biological response is limited to a very short period" (Stöckel and Thoenen 1975a [165]).
- In 1986, Godfrey and Shooter quantified that "[p75, lower-affinity receptors] are about 13-fold more numerous than the higher-affinity (TrkA) receptors" and that NGF was "initially bound to low-affinity (p75) receptors (Godfrey and Shooter 1986 [9]);
- Measuring the K_d (dissociation constants) for both receptors, Godfrey and Shooter also noted that 10%-20% of binding appeared to be non-specific, that "[t]he fraction of rapidly dissociating ^{125}I-βNGF increased with increasing concentration of ^{125}I-βNGF used for binding, and thus with increasing occupancy of "[p75, low-affinity] receptors" (Godfrey and Shooter 1986 [9]). Their report suggested the possibility that NGF binds first to low-affinity p75 receptors, then both, then mainly to high-affinity TrkA receptors. By 1997, the precise binding sequence to low and high receptors still had not been shown, but co-occurrence had been shown to be feasible (Dumas 1979 [166]; Olender and Stach 1980 [167]; Ure and Campenot 1997 [23]);
- Citing Battleman *et al.,* 1993 [168], Loy *et al.* speculated that p75 formed part of the NGF-TrkA complex (Loy 1994 a,b [169, 170]). The Battleman study was conducted with an HSV-1 vector:

  > "Previous equilibrium binding studies have indicated that p75^{NGFR} participates in high-affinity NGF binding" (Green and Greene 1986 [171]; Hempstead 1989 [172], Hempstead 1991 [94]; Pleasure 1990 [173]; Matsushima and Bogenmann 1991 [174]) and "[w]hether this binding requires the concomitant expression of p75NTR has been the subject of debate (Bothwell 1991 [175]; Chao 1992 [176]). See also Lee 1992 [177], Chao 1994 [149]."

- In 1996, von Bartheld *et al.* conducted an experiment of p75 and TrkB *in vitro*, in chick optic nerve (von Bartheld 1996 [178]). They "implicated" p75 (p75NTR), and made the case that low and high affinity receptors are important for retrograde axonal transport:

  > "Both p75NTR and trkA are transported retrogradely (Johnson 1987 [77]; Loy 1994 a, b [169, 170]; Ehlers 1995 [179]). Previous studies on adult and postnatal animals have implicated p75NTR as well as trk receptors as mediators of neurotrophin transport (DiStefano 1992 [137]; Yan 1993 [180]; Curtis 1994, 1995 [142, 181]).

2 STEP TWO: INTERNALIZATION (ENDOCYTOSIS) OF THE NERVE GROWTH FACTOR-TROPOMYOSIN KINASE A COMPLEX

The term endocytosis ("internalization") in the context of NGF means absorption at distal nerve endings. For NGF, internalization occurs at the distal ends (nerve endings). More broadly, endocytosed ("internalized") describes the process by which other non-neuronal proteins are endocytosed.

The process of internalization is known to be both physiological and signal enabled. This review focuses on the physiological internalization of NGF. It is known that NGF is "internalized," or loaded onto the retrograde axonal transport system via a surface-bound process, and that loading is a relatively efficient process, ~ 85%. The term "internalized" is used carefully since it can describe both the general processes of endocytosis and phagocytosis, and specifically the intraneuronal 'surface-bound' transport of the NGF/TrkA complex in microtubules. For a review of internalization, see Table 2 below, Xu 2017 [182]).

Microtubules are the rigid, non-lipid cyto-skeletal components of axons, each a hollow rod of about 25 nm (Cooper 2000 [183]). Intraneuronal transport in microtubules differs from, say, extraneuronal binding to myelin, the external sheath that surrounds peripheral axons with lipid-rich material (Morell and Quarles 1999 [184]).

2.1 Key points

It is definitive that -

(i) NGF is endocytosed in complex with either the TrkA and/or the p75 receptor(s);

(ii) NGF is probably transported in vesicles;

(iii) NGF has been previously modified (Table 3), resulting in larger complexes than even the small molecule fluorescent dye used in 800-rhNGF (rhNGF = 26.3 kDA + dye MW 1015), which complexes remained active after delivery, *i.e.,* endocytosis did not alter the larger construct; and

(iv) The efficiency of transport, once binding has occurred and the complex is loaded onto the retrograde axonal transport system, has been shown. As reported by Ure and Campenot in 1997, "[d]issociation assays indicated that 85% of ^{125}I-NGF associated with distal axons was surface-bound." This suggests that 85% was transported (Ure and Campenot 1997 [23]), and that after binding and endocytosis, transport is a relatively efficient process.

What is not definitive – with the possible exception of phosphorylation – are what signals are sent, by what molecular processes, and what factors like age affect internalization and retrograde transport of NGF. Nor is it clear what signals load the NGF-TrkA(-p75?) complex onto the retrograde axonal transport system. In 2009, three possible mechanisms of retrograde signaling were considered (Campenot 2009b [185]).

2.2 Internalization: definition of vesicles, endosomes, and lysosomes

Very broadly, endocytosis is a process by which a cell absorbs (imports) substances or molecules. This can occur in nerve and non-nerve cells (Straubinger 1983 [186]; Murthy 1998 [187]; McMahon 2011 [188]; Pryor 2012 [153]; Du Toit 2015 [189]; Xu 2017 [182]). Endocytosis is a general process that includes NGF. Generally, endosomes move retrogradely; lysosomes, including axonal autophagosomes, move anterogradely (Frampton 2012 [43]; Roney 2022 [190]). Endosomes are membrane-bound vesicles (Brown 2003 [191]), and may be early, mature (late), or transport recycled materials (Condon and Ehlers 2007 [192]; Gindhart and Weber 2009 [193]).

There are studies reporting that the NGF-TrkA complex is internalized or transported in vesicles (Ye 2003 [194]; Marlin and Li 2015; [195]; Lehigh 2017 [196]; Barford 2017 [197]), or are described as internalized in the endosomal-lysosomal system (Hu 2015 [198]). That endocytosis of the NGF-receptor complex occurs is not in dispute. What remains unclear is by what mechanism (Ye 2018 [199]).

2.3 Internalization: size (comparison of endosomes to lysosomes)

The literature confirms that various molecules may be conjugated to rhNGF:

- Table 2 compares endosomes vis-à-vis lysosomes, using size as a comparator.
- Table 3 then looks at the size of payloads that NGF might transport. Table 3 estimates the Molecular Weight (MW) and the apparent size - kilodalton (kDA is or 1/1000th of a nanometer, nm) - of various dye and drug payloads that have been conjugated to NGF.

  Table 3 converts MW and kDA to nm, enabling comparisons (nanoComposix FORTIS online calculator https://nanocomposix.com/pages/molecular-weight-to-size-calculator) (nanoComposix FORTIS [200]; Muster 1980 [201]; Erickson 2009 [202]). The designation nanometer is nm, or 1/1000th of micrometer, which is designated μm.

For example, NGF is estimated to be a dimer of MW 26.3 kDA. Using the nanoComposix online calculator, the nm equivalent is 3.94 nm. This places rhNGF into the clathrin-dependent endocytosis (CDE) category. This review does not attempt to reconcile either the names or the forms of NGF (murine or recombinant human NGF; High Molecular Weight (HMW), 2S or 7S, or beta-rhNGF) (L.E. Burton, personal communication)..

Endocytosis may be divided as to size and function: when endocytosis is clathrin-dependent, it involves pinocytosis. By comparison, "[p]hagocytosis implies the ingestion of large and solid particle (diameter 0.5–10μm) such as pathogens" (Xu 2017 [182]; Paul 2013 [203]; Baranov 2020 [204]). How and that clathrin-coated vesicles are endocytosed has been known for decades; they are known to be highly conserved evolutionarily. In addition to NGF (Howe 2001 [25]), clathrin-dependent endocytosis applies to at least 50

molecules (Kaksonen and Roux 2018 [205]; Xu 2017 [182]; Bronfman 2003 [155]; Ye 2018 [199]; McMahon 2011 [188]; Du Toit 2015 [189]; Xu 2017 [182]).

Table 2. Comparison of endosome vs lysosome
Non-phagocytotic (pinocytotic) categories of endocytosis (source: Xu 2017 [182])

Endosome		Lysosome
Retrograde (periphery to neuronal cell body-CNS)		Anterograde (neuronal cell body-CNS to periphery)
Pinocytosis		Phagocytosis
(smaller)		(larger)
Macro-pinocytosis 0.1-5μm (100 nm - 5000 nm); Clathrin-dependent < 120 nm; Caveolin-dependent <80 nm; Clathrin-caveolin independent <50 nm		Particle diameter 0.5-10μm
Vesicle (membrane-bound)	Non-membrane-bound	
Formed during internalization		Formed from Golgi apparatus
Degraded by proteolysis		Degraded by hydrolytic enzymes
		May fuse with endocytosis (myosin V “recycle”)

Table 3. Size of payloads: rhNGF: receptors, radiolabel, commercial small molecule, other
(calculation sources: Erickson 2009 [202]; nanoComposix Fortis [200])

Ligand/-receptor or conjugate	Estimated Size (nm)	Reported size MW/kDA: NGF, radiolabel, small molecule dye, or drug payload	Reference
rhNGF	3.94	Monomer 13.6 kDA (dimer 26.3 kDA)	Frey 1997 [206]
^{125}I	0.663	Iodination label MW 125.9126	Frey 1997 [206]
TrkA	6.92	Monomer 85 kDA (dimer 143 kDA)	Hosang and Shooter 1985 [40]
rhBDNF	3.98	Monomer 13.5 kDA (dimer 27.3 kDA)	BDNF and TrkB have multiple isoforms (Mandel 2009 [46]; Rosenfeld 1995 [207])
TrkB	6.87	Glycosylated 140 kDA	Silhol 2005 [208]; Otani 2017 [209]
p75	5.76	80-85 kDA	Chao 1986 [108]; Johnson 1986 [109]; Radeke 1987 [110]; Rodriguez-Tebar 1992 [68]
800-rhNGF	3.94+1.33	Fluorescent dye MW 1,050	Manzanita Pharmaceuticals: attached directly to NGF (no linker)
NGF-OX-26	3.94+3.45	OX-26 95-150 kDA (OX-26 is the antibody to the transferrin receptor, known to cross the blood-brain barrier, here to enable NGF to reach the CNS)	Kordower 1994 [210]; Charles 1996 [211]; for OX-26 only, see Yue 2012 [212]
NGF-AAV2	3.94*+3.45 **pro forma*	AAV2 MW 150 kDA (Pillay 2017 [213])	Tuszynski 1996 [214]; Granholm 1998 [215]; Tuszynski 2006 [216]; Tuszynski 2007 [217]; Nagahara and Tuszynski 2011 [218]; Bartus 2012 [219]; Bartus 2013 [220]; Rafii 2014 [221]; Rafii 2018 [222]; for AAV2, see Muster 1980 [201]; for all AAV, see Govindasamy 2013 [223]
			See also amphotericin for Candidal meningitis Tang 2015 [224]; see also stroke Amani 2019 [225]

Results in Table 3 make reasonable biological sense, and confirm the approach of applying the nanoComposix online calculator to all estimates. Assuming the estimates from the nanoComposix calculator and other methods are plausible, the examples below would all “fit” into microtubules (25 nm):

- **NGF-TrkA.** Using a Stokes Radius approach, where S = sedimentation co-efficient, or Stokes Radius; assuming the High(est) Molecular Weight (HMW) complex is 7S NGF (5 x 26K molecules, one of which is dimeric 26K total 7S ~ 130K; Stokes Radius would calculate 2.8X. Since actual weight ratio is closer to 5Xm solving for R (radius), taking weight (4/3 x pi x R^3), the ratios of the two “R’s (*radii*)” are almost the same as the Stokes Radius ratios (approximately 1.78 vis-à-vis 1.7).

Recall that the NGF receptor complex dimerizes, where the NGF dimer is "sandwiched" between the two TrkA receptors. This is where the different methods used to calculate MW and Stokes Radius matter. For example, the monomer weight for NGF used SDS page gels (not globular). The AAV size calculation uses kb to estimate the size of genes (Govindasamy 2013 [223]).

- **NGF-AAV2 construct.** Importantly, the NGF-AAV2 construct is not a conjugate of NGF-AAV2. Rather, the NGF-AAV2 construct inserts the NGF gene – not the NGF-receptor complex - into AAV2. AAVs are 4.8 kb; the MW of AAV2 is 150 kDA (Pillay 2017 [213]).
- **NGF-OX-26 conjugate.** Since transferrin crosses the blood-brain barrier, but NGF does not, the strategy arose to conjugate the two. Another group derivatized rhNGF by conjugating it to the anti-transferrin receptor antibody, OX-26. "This conjugation facilitates the transfer of NGF from the systemic circulation to the CNS via the transferrin transport system" (Charles 1996 [211]). Depending on the species from which it is derived, the MW of OX-26 is roughly 95-150 kDA. Bartus *et al.* modified NGF to create an NGF-OX-26 conjugate (Kordower 1994 [210]; Charles 1996 [211]; for OX-26 only, see Yue 2012 [212]).
- **Labelling NGF with iodine.** We considered iodinated rhNGF used in primate studies. Radiolabeled iodine adds MW 125.9126 to NGF. The selectivity of iodinated sub-cutaneous (SQ) rhNGF for TrkA-expressing sensory neurons was quantified in non-human primates (Nguyen 2000a [226]). In a study of repeat dose administered sub-cutaneously rhNGF, about 80% of iodinated NGF was absorbed by sensory neurons near the injection site (Nguyen 2000 a, b [226, 227]). That report does not include analysis of the rate of label instability.

2.4 Internalization: phosphorylation and signal transduction pathways

It is known that phosphorylation occurs within minutes of TrkA binding, and promotes endocytosis. Phosphorylation adds a phosphoryl group (PO_3) to the serine, threonine, or tyrosine residues of a protein. By 1991, studies of NGF phosphorylation in PC12 cells identified phospholipase C gamma-1 (PLC-γ1) as the key phosphoryl group (Kaplan 1991b [228]; Kim 1991 [229]; Vetter 1991 [230]. In compartmented cultures, tyrosine NGF phosphorylation begins almost immediately, very soon after NGF binds to TrkA (Treanor 1995 [30]; Senger and Campenot 1997 [10]; Butowt and von Bartheld 2009 [31]; Lehigh 2017 [196]).

In 1997, Senger and Campenot reported that the initial phosphorylation of NGF-TrkA subsequently induced phosphorylation of several, secondary messenger proteins (Loeb 1991 [99]; Loeb and Greene 1993 [100]; Ibáñez 1992 [101]; Kaplan 1991a, b [228, 231]; Klein 1991a [81]; Jing 1992 [232]; Senger and Campenot 1997 [10]). This observation further strengthened the importance of the location of TrkA receptors at distal ends (Campenot 1977 [115], 1982a [117], 1987 [116]; Campenot 1994 [118]).

There is no consensus as to whether vesicle-mediated and/or non-vesicle-mediated signal transport are involved with phosphorylation (Campenot 1994 [118], Curtis and DiStefano 1994 [181], DiStefano and Curtis 1994 [141]; Watson 1999 [233]; Miller and Kaplan 2001 [234]; Neet and Campenot 2001 [8]; Sofroniew 2001 [235]). Neither is there consensus as to the possible mechanisms of retrograde signaling (Campenot 2009b [185]). Of three models proposed (Howe 2001 [25]; Hendry and Crouch 1993 [236]; Campenot 1994 [118]), Campenot noted that two "regulate neuronal survival" (Campenot 2009 [185]).

Through most of the 1980s, it was believed that "(retrograde) transport of ….. [signaling molecules was] essential for the survival of developing neurons" (Purves 1988 [237]; Barde 1989 [238]; Zweifel 2005 [239]; Oppenheim and von Bartheld 2008 [240]; Butowt and von Bartheld 2009 [31]). The notion that signaling and physiological transport could be distinct was suggested in 1996 (von Bartheld 1996 [178]). Then in 2002, MacInnis and Campenot conducted a series of elegant experiments and reported that "….[an NGF-TrkA] neuronal survival signal can reach the cell bodies unaccompanied by the NGF that initiated it" (MacInnis and Campenot 2002 [241]). This distinction was subsequently noted by others (Chao 2003a [150]; Chao 2003b [242]; Mok 2007 [243]). In other words, the retrograde axonal transport of NGF-receptor complex is probably physiological, but some of that axonal transport may also be due to signaling.

Finally, NGF-TrkA signaling activates several signal transduction pathways that have been identified as critical in multiple functions including neoplasticity and analgesia, including the Ras/MAPK pathway; the PLC-γ1 pathways (this section, first paragraph); the pathway that activates the capsaicin receptor Transient Receptor Potential Vanilloid 1 (TRPV1) (Caterina 1997 [244]) via the phosphoinositide-3-kinase (PI3K) and mitogen activated protein kinase (MAPK), but not the phospholipase C (PLC) pathway (Zhu and Oxford 2007 [245]) or

the PI3K/Akt-mTOR pathway; and the p75NTR-mediated signaling pathway. This review does not discuss signal transduction pathways other than phosphorylation.

2.5 Endocytosis: conclusions

The evidence is conclusive, that (i) all neurotrophins, including NGF, undergo endocytosis after binding; and (ii) NGF can be endocytosed, in conjunction with either small molecule or larger payloads (Table 3). What remains to be investigated are whether physiological and/or various signals are required for endocytosis to occur; and whether primary and/or secondary phosphorylation messengers are involved.

As recently as 2022, the issues of signaling and/or physiological retrograde axonal transport had not been fully clarified (Conroy and Coulson 2022 [246]). The authors note that

> " … there is substantial evidence that [p75 and TrkA] work together to enhance trophic signaling during development and in the healthy brain by mediating at least 10-fold higher affinity of the Trk receptor for its cognate neurotrophin" (Conroy and Coulson 2022 [246]).

The lack of conclusiveness is not surprising, since it seems partly due to lack of clear and/or consistent terminology; partly because "signaling endosomes" have such a wide breadth of neuronal functions, including neuronal survival and changes in gene expression (Hamburger 1949 [2]; Cohen 1954 [3]; Levi-Montalcini, 1976 [4], Levi-Montalcini 1987 [247], Levi-Montalcini 1996 [248]; Mathew and Miller 1990 [249]; Miller 1991 [250]; Ma 1992 [251]; Wyatt and Davies, 1995 [252]; Toma 1997 [66]; Bibel and Barde 2000 [253]; Poo 2001 [254]; Chao 2003b [242]; Huang and Reichardt 2003 [255]); partly because studies of late endosomes and endosomal-lysosomal fusion relied on different models, of differing species, whose axons are shorter or longer (Burton P.R. and Paige 1981 [256]; Heidemann 1981 [257], 1984 [258]; Yano and Chao 2000 [259]; Delacroix 2003 [260]; Miaczynska 2004 [261]; Heerssen 2002 [262]; partly due to the multiple pathways that have been elucidated (Wu 2007 [263]; Mitchell 2012 [264]; Courchet 2013 [265]; Greif 2013 [266]; Zala 2013 [267]; Marlin and Li 2015 [195]; Hinckelmann 2013 [268]; 2016 [269]; Barford 2017 [197]; Ye 2018 [199]; Sainath 2021 [270]); and partly due to the variable maturation of NGF-containing vesicles prior to retrograde axonal transport, as has been described for epidermal growth factor and other molecules (Dunn and Hubbard 1984 [271]; Schmid 1988 [272]; Stoorvogel 1991 [273]; Ure and Campenot 1997 [23]).

Finally, to our knowledge, there is no model of binding, endocytosis, or axonal transport in which the variables of length, age, species, nerve groups, and/or *radii* of axons are tested. A review in 1980 highlighted the complexity of this issue: in immature animals the velocity of slow transport appears to be two-to-three times faster than in adults, whereas the velocity of fast transport increases two-to-three times during the course of maturation, with the time of arrival of the most rapidly transported material at the axon terminals remaining approximately constant despite axonal elongation (Grafstein and Forman 1980 [274]).

3 STEP THREE: AXONAL TRANSPORT – RETROGRADE AND ANTEROGRADE

In reviewing the original experiments involving NGF and other proteins that measure the velocities of retrograde and anterograde axonal transport (Appendix C), it is important to note that velocities may be "reasonable biologically," but are not precise. It also appears that most of the basic research into velocities of fast axonal transport were done in the 1980s (see multiple papers and book chapters by Brady *et al*.), and slow retrograde axonal transport – by Campenot *et al.* in the late 1990s.

However, variability is also reasonable. Intra- and inter-group variability may be due to differences in approaches – different *in vitro* bioassays, different *in vivo* species, and different nerve groups – that were used in the 1970s, 1980s, and 1990s when original studies were done. This review does not 'harmonize' or otherwise try to reconcile the differences in reports.

3.1 Key points

It is definitive that –

(i) Axonal transport includes NGF and other proteins, where axonal transport enables metabolism, specifically in the case of NGF, neuronal metabolism;

(ii) There are fast and slow components of both directions of axonal transport;

(iii) The fast axonal transport component includes the anterograde ("orthograde") direction;

(iv) Both components – fast and slow – of axonal transport are bi-directional, beginning in the retrograde direction, retrograde axonal transport (from the periphery to the neuronal cell body), then returning in the anterograde direction, anterograde axonal transport (from the neuronal cell body at the center, back to the periphery) after degradation; and

(v) For NGF specifically, after retrograde axonal transport, NGF is degraded mainly by proteases, nucleases, esterases, glycosidases, lipases, phosphatases, and sulfatases (Avers 1982 [37]; Sheeler and Bianchi 1983 [38]; Parton and Dotti 1993 [39]; Hosang and Shooter 1986 [40]; Vissavajjhala 1992 [41]; Neet and Campenot 2001 [8]; Boutilier 2008 [42]; Frampton 2012 [43]). *In vitro* studies found that slow retrograde axonal transported NGF has a half-life of 6h (Ure and Campenot 1997 [23]; review, Grafstein and Forman 1980 [274]).

3.2 Retrograde axonal transport: definition

Retrograde axonal transport occurs in all neuronal types, not only in sensory neurons, but also in motor neurons. Retrograde means the movement of cargo by neurotrophins from the distal ends or nerve endings (axon terminals) at the periphery, to the neuronal cell bodies, or soma.

Retrograde transport was observed early, in 1948 by Weiss and Hiscoe (Weiss and Hiscoe 1948 [275]). After its discovery, retrograde axonal transport was widely studied in the 1970s and 1980s, first with a known neurotracer, horseradish peroxidase (HRP) (LaVail and LaVail 1972 [276]; Honig and Hume 1989 [277]), and with NGF (Kristensson 1971 [278]; Hendry 1974a, b [32, 164]; Stöckel 1974 [121]; Iversen 1975 [127]; Stöckel and Thoenen 1975 [88]; Schwab and Thoenen 1977 [279]; Johnson 1978a [76]; Dumas 1979 [166]; Schwab 1979 [280], 1980 [281]; Thoenen and Barde 1980 [282]; Claude 1982b [283]; Schwab 1982 [284]; Bisby 1982 [285]; Schwab 1983 [286]; Korsching and Thoenen 1983a [287]; Palmatier 1984 [90]; Ross 1994 [288]).

3.3 Retrograde and anterograde axonal transport: reported velocities

Fast and slow retrograde axonal transport. Table 4 lists *in vitro* studies and *in vivo* studies, so that a rough comparison may be made. As discussed in Appendix C, both slow retrograde axonal transport (10-20 mm/hr) and the slow component of fast anterograde axonal transport exist. The velocity of fast retrograde axonal transport is reported as ten times (10X) faster, or 100-200 mm/hr or 200-400 mm/hr (Vallee and Bloom 1991 [289]; Ure and Campenot 1997 [23]). However, retrograde axonal transport has also been reported using neurotracers, for example, HRP (LaVail and LaVail 1972 [276]) or fluorescent tracers (Honig and Hume 1989 [277].

- Using ^{125}I-NGF to quantify uptake in compartmented cultures, in 1997 Ure and Campenot quantified slow retrograde axonal transport *in vitro*, reporting the velocity of slow retrograde axonal transport as 10-20 mm/h (Ure and Campenot 1997 [23]). In that study, higher, faster transport rates were recorded in younger cultures. Reports are inconsistent as to the influence of the age of receptors on study outcomes.

  > "This [rate of 10-20 mm/hr] is higher than the estimate of 2–3 mm/hr made for NGF retrograde transport in adult rat sympathetic neurons *in vivo* (Hendry *et al.,* 1974 a,b; Johnson *et al.,* 1978), but it is similar to estimates of 7–13 mm/hr for NGF transport by sensory neurons (Stöckel *et al.,* 1975; Yip and Johnson, 1986) and 12 mm/hr for dopamine b-hydroxylase transport in sympathetic axons of the sciatic nerve (Brimijoin and Helland, 1976)."

- Slow retrograde axonal transport begins 30-60 min after application to distal ends (Ure and Campenot 1997 [23]; Senger and Campenot 1997 [10]). Compared to other proteins, this is a relatively slow rate. However, slow retrograde axonal transport alone cannot explain the rapid absorption of 800-rhNGF, when 800-rhNGF was consistently taken up within the 15-30 min specified for clinical utility. The slow rate of retrograde axonal transport supports the plausibility of durable signal ('what the surgeon sees');

- Fast retrograde axonal transport is estimated to be 100-200 mm/h, about 10X – 20X faster than slow retrograde axonal transport. The faster rate provides an explanation for the rapid absorption of 800-rhNGF in nonclinical studies (Campenot and Eng 2000 [290]; Campenot 2003 [291]; Gibbs 2015 [292]; Sleigh 2016 [293]); and

- As summarized below in Table 4, *in vivo* studies of the slow rate of retrograde axonal transport of between 2.5 mm/h – 13 mm/h (Stoeckel and Thoenen 1975a, Stoeckel and Thoenen 1975b [88, 294]; Johnson 1978a [76]; Yip and Johnson 1986 [295]) correspond roughly (within "reasonable biological range") to previously reported *in vitro* findings of 10-20 mm/h (Hendry 1974a, b [32, 164]; Brimijoin and Helland 1976 [33]; Ure and Campenot 1997 [23]).

Table 4. Comparison of *in vitro* to *in vivo* rates of slow retrograde axonal transport (1974-1997)

In vitro				
	10-20 mm/h		*In vitro,* compartmented cultures superior cervical ganglia, **neonatal rat**	Ure and Campenot 1997 [23]
NGF a, NGF b	(a) 2.5 mm/h (b) 2.2-2.5 mm/h		*In vitro*, a – anterior intra-ocular injections, **mouse**; b – anterior intra-ocular injections, **rat, mouse**	Hendry 1974a, b [32, 164]
dopamine-β-hydroxylase	12mm/h		*In vitro*, sciatic nerve, **rabbit**	Brimijoin and Helland 1976 [33]
NGF	3 mm/h		*In vitro,* compartmented cultures, superior cervical ganglia, **neonatal rat**	Claude 1982b [283]
In vivo				
NGF a, NGF b		(a) 2.5 mm/h (b) 13 mm/h	*In vivo*, a – anterior intra-ocular injections in superior cervical ganglia and submaxillary gland, **mouse**; b – injected forepaw, **rat**	Stoeckel a, b 1975 [88, 294]
NGF		3 mm/h	*In vivo*, a – anterior intra-ocular injections, **rat, hamster**	Johnson 1978a [76]
NGF, HRP		7 mm/h	*In vivo*, crushed sciatic nerve into DRG, **rat**	Yip and Johnson 1986 [295]

Fast retrograde axonal transport: 200-400 mm/da. Fast retrograde axonal transport occurs at about 200-400 mm/da, and can be retrograde (from the periphery, toward the cell bodies) or anterograde (orthograde, from the cell body after degradation, toward the periphery) (review, Grafstein and Forman, 1980 [274]; Allen 1982 [34]; Brady Lasek and Allen 1982 [296]; Brady 1985a [297]; Brady 1985b [29]; Brady 1984 [27]; Brady 1991 [298]; Brady 1993 [28]; Bloom 1984 [299]; Paschal and Vallee 1987a [300]; Paschal 1987b [301]; Leopold 1990 [302]; Stenoien and Brady 1999 [303]; Aboud 2015 [304]). Fast axonal transport is mediated in part by the low affinity, pan-neurotrophin receptor p75 (Yankner and Shooter 1979 [305]; Landreth and Shooter 1980 [306]; Schechter and Bothwell 1981 [307]; Hosang and Shooter 1985 [40]).

It is generally accepted that differences in rates of fast or slow retrograde axonal transport can be explained partly or mainly by differences in cargo size and cargo binding affinities (Roy 2014 [47]). Fast axonal transport is probably membranous ("***faster moving materials were associated with membranous structures***") (Brady 1985b [29]), occurring in vesicles, where vesicles are enclosed in a membrane (Grafstein and Forman 1980 [274]; Lasek and Brady 1982 [308]; Tytell 1981 [309]; Baitinger 1982, 1983 [310, 311]).

Table 5. Fast & Slow Axonal Transport
(sources: Brady 1985b [29] and Brady 1991 [298])

Rate component	Rate mm/day	Examples of protein composition	Axonal cytological structure
Fast axonal transport			
Fast axonal transport	50-400	Membrane-associated materials	Membranous organelles
Orthograde	200-400	Na/K-ATPase, transmitter-associated enzymes, and GAPs	Sodium-potassium ATP energy pump; 5-nm tubulo-vesicular structures, dense-core vesicles
Mitochondria	50-100	F_1-ATPase, small amount of spectrin	Mitochondria
Retrograde	200	Lysosomal hydrolases, NGF, other materials obtained by endocytosis	Prelysosomal structures (multi-vesicular, multi-mellar bodies)
Slow axonal transport (slow component of fast axonal transport)			
Slow	0.1-6	Cytoskeletal, associated proteins	Cytomatrix
SCb *	2-6	Actin, clathrin, spectrin, NSE, CK, calmodulin, aldolase, pyruvate kinase	Microfilaments, cytoplastic matrix
SCa **	0.1-1.0	Tubulin, neurofilament triplet, tau proteins, spectrin	Microtubule-neurofilament network
SCb * = slow component b, anterograde (Elluru 1995 [312])			
SCa ** = slow component a, anterograde (Elluru 1995 [312])			
Key: Na/K-ATPase = Na+/K+ ATPase (Pirahanchi StatPearls 2023 [313]); GAP = GTPase associated proteins; F_1-ATPase = Xu 2015 [314]; NSE = nerve specific endolase; CK = creatine kinase, also creatine phosphokinase			

The distinction between membrane-associated vesicles is made by Brady (Brady 1985b [29]): "it has been shown that membrane-associated activities in the axon are dependent on fast transport, whereas changes in the cytoskeleton and many metabolic activities can be related to slow transport (Grafstein and Forman, 1980;

Lasek and Brady, 1982a).” One report attempted to reconcile the “vesicles vis-à-vis non-vesicles” debate, and found that there are forms of retrograde transport, *i.e.* TrkA-mediated slow retrograde transport, that are non-membranous (Brown 2003 [191]).

Anterograde axonal transport. Finally, moving in the other direction from cell body to periphery, is anterograde (“orthograde”) axonal transport, which has a velocity of about 1 mm/sec (Twelvetrees 2016 [315]; Guillaud 2020 [316]). Like retrograde axonal transport, anterograde axonal transport has a fast and slow component (Dillman a, b 1996 [317, 318]). In 1996, Dillman *et al.* found that 80% of anterograde axonal transport is slow, but only ~15% is fast (Elluru 1995 [312]; Dillman 1996b [318]; Tytell 1981 [309]; Brady 1985b [29]; Lasek 1986 [319]; Vale 1992 [320]). In 1987, Johnson found that anterograde transport was not merely the mirror image of retrograde transport: “… staining on the distal side … was concentrated in granular or short fusiform (punctate?) configurations, whereas on the proximal side … the staining was distributed in longer attenuated figures, as in a stream” (Johnson 1987 [77]).

The complexity of anterogradely transported NGF after degradation is known (Stenoien and Brady 1999 [36]):

> “Anterograde transport provides newly synthesized components essential for neuronal membrane function and maintenance. Ultrastructural studies have demonstrated that the material moving in fast anterograde transport includes many small vesicles and tubulovesicular structures as well as mitochondria and dense core vesicles (Smith 1981 [321]; Tsukita and Ishikawa 1980 [322]) …. Material in fast anterograde transport is needed for supply and turnover of intracellular membrane compartments (mitochondria and endoplasmic reticulum), secretory products and proteins required for the maintenance of axonal metabolism. The net rate appears to be largely determined by size, with the smallest MBOs (Membrane Bound Organelles) in almost constant motion, while mitochondria and larger structures frequently pause, giving a lower average rate (Brady 1985b, Brady 1985c [29, 323]).
>
> “A variety of materials move in fast anterograde transport, including membrane-associated enzymes, neurotransmitters, neuropeptides and membrane lipids. Most are synthesized in the cell body and transported intact, but some processing events occur in transit. For example, neuropeptides may be generated by proteolytic degradation of pro-peptides ….. This biochemical heterogeneity extends to the MBOs themselves. The small organelles are particularly varied in function and composition: some correspond to synaptic vesicle precursors and contain neurotransmitters and associated proteins, while others may contain channel proteins or other materials destined for the axolemma. Biochemical and morphological studies have provided a description of the materials transported in fast transport but are not as well suited for identifying the underlying molecular mechanisms involved in translocation” (Stenoien and Brady 1999 [36]).

3.4 Axonal transport: molecular motors

The chemico-mechanical mechanisms (“molecular motors”) of retrograde and anterograde axonal transport for NGF are dynein, kinesin, and myosin V. Historically, axonal transport was studied not only for its bi-directionality (retrograde and anterograde) and velocity, but also for the ability to quantify and/or reveal the receptors involved (TrkA and/or p75) and/or the molecular motors involved (dynein, kinesin, myosin V).

The molecular motors powering axonal transport are not considered other than as background to retrograde axonal transport. One report found that “the main difference between fast and slow transport seems to be related to ‘duty-ratio’; the proportion of time that cargo-structures spend moving” (Roy 2014 [47]).

- **Dynein** mainly enables retrograde axonal transport, traveling from nerve endings to the neuronal cell body, where the minus-end is located (Paschal 1987 [324]; Schnapp and Reese 1989 [325]; Bhabka 2016 [326]; Can 2019 [327]; Canty 2021 [328]; Dillman 1996a [317]). However, as noted by Dillman *et al.* (Dillman 1996b [318]), dynein is also one of the two (fast and slow) components of anterograde transport: “… transport of membranous organelles from the synapse to the cell body” (Vallee and Bloom 1991 [329]; Brady 1991 [298]; Vale 1992 [320]; Iqbal 1991, Bunke 1991 [330, 331]).
- **Kinesin** mainly enables anterograde transport. However, recent studies suggest that the two molecules dynein and kinesin can work together, possibly linking slow and fast axonal transport (Paschal 1987 [324]; Tang 2013 [332]; Roy 2014, 2020 [47, 333]; Reck-Peterson 2018 [334]); and
- **Myosin V** transports cargo on actin tracks, not on or in microtubules (Mehta 1999 [335]; DePina 1999 [336]). It also plays a role in axonal transport of motor neurons (Coureux 2003 [337], 2004 [338]; Trybus 2008 [339]). In sensory neurons, during the transport of kinesin-bearing cargoes, myosin V reduces and even stops movement when myosin V and kinesin occur equally (Mehta 1999 [335]; DePina 1999 [336]; Pathak 2010 [340]; Goldman 2012 [341]; Janssen 2017 [342]).

3.5 Retrograde axonal transport: safety ('steady state;' location of site of degradation; half-life; efficiency of uptake)

Finally, we are interested in four pharmacokinetic parameters of NGF, all of which directly and indirectly affect safety: (i) 'steady state,' when TrkA receptors become saturated; (ii) efficiency of uptake; (iii) location of the site of degradation; and (iii) half-life, or rate of degradation after transport of NGF retrogradely to the neuronal cell body, after which it is returned to the periphery in anterograde axonal transport.

We consider several factors in evaluating the rate of retrograde axonal transport, which rate influences the local ADME (Absorption, Distribution, Metabolism, Excretion). The ADME parameter is critical to surgeon and patient safety, since we expect NGF to remain in tissue for roughly 6h (Ure and Campenot 1997 [23]), *i.e.,* after a surgical procedure is concluded, and after the patient is sutured up.

3.5.1 'Steady state' reflects saturation of TrkA receptors. Among the factors representing rate of transport, an important concept is saturation of receptors. When TrkA receptors are saturated, they reach a 'steady state,' transporting 2%-25% of distal axon-associated NGF each hour (Ure and Campenot 1997) [23]. Note that this 1997 report did not consider saturation of p75 receptors.

3.5.2 Efficiency of uptake: approximately 80%. In that same study by Ure and Campenot, the post-binding efficiency of NGF-TrkA was quantified, the amount that reaches the cell body after binding and after retrograde axonal transport (Ure and Campenot 1997 [23]). Once bound, an estimated 85% of iodinated NGF reaches the neuronal cell body "[i]n cultures allowed to reach steady state ^{125}I-NGF transport, cell bodies contained only 5–30% of the total neuron-associated ^{125}I-NGF, whereas 70–95% remained associated with the distal axons" (Ure and Campenot 1997 [23]).

3.5.3 Mechanism and locations of sites of degradation of NGF and the NGF-TrkA complex. After transport, NGF is degraded in the neuronal cell body (Butowt and von Bartheld 2009 [31]; Rind 2005 [343]). Upon arrival at the neuronal cell body, the NGF-receptor complex is degraded by proteolysis, when the endosomes containing NGF bound TrkA fuse with lysosomes containing nucleases, proteases, esterases, glycosidases, lipases, phosphatases and sulfatases (Avers 1982 [37]; Sheeler 1983 [38]; Parton and Dotti 1993 [39]; Hosang and Shooter 1986 [40]; Vissavajjhala 1992 [41]; Neet and Campenot 2001 [8]; Boutilier 2008 [42]; Frampton 2012 [43]).

3.5.4 Half-life of NGF *in vitro* is approximately six (6) hours. After binding, *in vitro*, the half-life of NGF after retrograde axonal transport is approximately 6h:

> "[a]fter transport, ^{125}I-NGF was degraded with a half-life of 6 hr. In summary, although some cellular events promoted NGF accumulation in cell bodies, distal axons represented by far the principal site of NGF receptor interaction at steady-state as a result of a low retrograde transport rate" Ure and Campenot 1997 [23].

The half-life of 6h reflects the rate of retrograde axonal transport – representing a relatively slow rate of degradation when compared to other proteins in non-neuronal cells (Chen 1982 [344]; Huang 1982 [345]; Wakai 1984 [346]; Davies 1985 [347]; Fujii 1986 [348]; Zoon 1986 [349]; Roupas and Herington 1987 [350]; Sorkin 1991 [351]; Yanai 1991 [352]; Auletta 1992 [353]; Nielsen 1992 [354]; Pandey 1992 [355]; Zapf 1994 [356]). Ure and Campenot speculate that, since Fibroblast Growth Factor (FGF) and Tumor Necrosis Factor-alpha (TNF-a) and their receptors are "internalized and degraded at different rates (Pennica 1992 [357]; Gleizes 1995 [358]), perhaps the degradation rate for NGF is different, depending on whether NGF is bound to [TrkA] or p75" (Ure and Campenot 1997 [23]). This suggests not only that the degradative half-life of the NGF-TrkA-p75 complex is affected, but also that NGF binds to one or the other or both.

4 SUMMARY

4.1 Future publications. In a future publication, we expect to report nonclinical results in rat (n=103, including prostate, facial nerve, axillary, and sciatic nerve), to include:

(i) Dose range-finding studies (conclusion: 1.0 mg/mL for Dye-Adduct-Ratio DAR2);

(ii) Signal-to-Background Ratio (SBR) calculations from nerve-to-muscle measurements which as expected reflect 'steady state,' as the larger DAR2 variant was ~ 25% higher than DAR1; and

(iii) Histological studies using an established co-localization approach, we confirmed TrkA as the binding mechanism for 800-rhNGF.

We will also report results from the GLP toxicology study in the second, canine mammalian species. Importantly, results in rat and dog may be considered clinically predictive. The TrkA binding phenomenon is highly conserved in mammals, including in humans (NCBI; KEGG, Genomes ([12, 13, 359]).

4.2 Productizing the science: 'what the surgeon sees.' It is known that NGF-TrkA is transported intraneuronally, but the NGF-receptor complex is 'surface-bound,' or travels in the lumina, on the outside of the cytoskeletal structures that are microtubules (Peters 1968 [20]; Rodriquez Echandia 1968 [21]; Burton P.R.1984 [22]; Ure and Campenot 1997 [23]; Garvalov 2006 [24]). This means that the dye signal needs to be sufficiently strong, that it can be visualized through the microtubules: the surgeon's eye and video display will 'see' only dye.

Further, the intraneuronal transport of NGF may be relevant insofar as microtubules represent a much lower lipid amount than extra-neuronal structures such as myelin. For example, it is the NGF component in the 800-rhNGF conjugate that binds to distal nerve endings, thus quantifying the PK of its intraneuronal absorption.

4.3 Appendix B: not explained, not tested. Appendix B lists those aspects of NGF science that we do not intend to investigate. For example, we have not, and do not intend to test systemic, intravenous (IV) injection of the 800-rhNGF conjugate. What can be surmised from the science is the following:

- When dye is attached to NGF, the dye is bound such that after wash, roughly 80% of 800-rhNGF continues to be absorbed. In other words, once bound to key localizing receptors, the saline wash does not destroy the continued binding, nor internalization, nor axonal transport of the agent;
- What nerves are illuminated are a direct function of what TrkA and/or p75 receptors are exposed and bound, or the spatial distribution pattern of these receptors. For example, in the surgical procedure of radical prostatectomies, only those receptors that are exposed in the peri-prostatic space can be bound. This means that 800-rhNGF may miss neurons that remain buried, because the nerve endings of those nerves are not exposed. We have not tested the re-application of 800-rhNGF to newly exposed nerves at the end of the procedure, but, subject to safety, in principle, re-application is possible.
- To our knowledge, there are no 'maps' of either TrkA or p75 in the peri-prostatic region of men. The absorption of NGF may be quantified using radiolabeled techniques; selective absorption is relatively efficient both *in vitro* (Ure and Campenot 1997 [23]) and *in vivo* (Nguyen 2000 [226]. Since NGF produces a 'death signal' soon – within 1-4h - after NGF withdrawal or deprivation (Campenot 1981 [360]; Seeley and Greene 1983 [361]; Campenot 1987 [116]; Martin 1988 [362]; Deckwerth and Johnson 1993 [363]), cadaveric studies of 800-rhNGF are infeasible;
- Every batch of 800-rhNGF was biologically characterized - tested for neuronal survival - prior to use *in vivo* in nonclinical studies. Neuronal bioassays use neonatal rats; rat studies use adult, healthy animals, and canine studies are done only in very aged, adult male animals. What is unknown is whether the age of receptors correlates with the age of the animal. This idea is important for example in the radical prostatectomies on US men - about 50% of whom are over 65: because the receptors for the at-risk cavernous nerves have been hidden, is the age of receptors reflected in the age of patients?;
- Preliminary stability studies confirmed the identity of the conjugate. These stability studies were conducted at various times, but only after rat experiments *in vivo* showed successful selectivity; and
- Finally, although in principle such a use is feasible, this review does not consider the utility of 800-rhNGF as an in-life neuro tracer (Lanciego and Wouterlood 2011 [364]).

4.4 Defective axonal transport is a hallmark of neurodegenerative disease. Finally, defective axonal transport has been implicated in many neurodegenerative diseases. Although reviewing pathology is not the goal of this review, very generally, after 2004 (Campenot and MacInnes 2004 [365]), most investigations of NGF focused on disease - on the pathology of defective NGF, phosphorylation, TrkA and/or defective p75, or defective molecular motors *e.g.* dynein, kinesin and/or myosin V.

There is a literature describing deficits in NGF-related axonal transport in several neurodegenerative, genetic, and even viral diseases, for example, including but not limited to:

- Multiple sclerosis (Sorbara 2014 [366]; van den Berg 2017 [367]; Guo 2020 [368]);

- Alzheimer's disease (Bunke 1991, Iqbal 1991 [330, 331]; Papasozomenos and Su 1991 [369]; Crutcher 1993 [370]; Goedert 1989 [371]; Goedert 1986 [89]; Higgins and Mufson 1989b [372]; Loy 1980 [373]; Phillips 1991 [374]);
- Amyotrophic lateral sclerosis (ALS) (Figlewicz 1994 a, b [375, 376]; Collard 1995 a, b [377, 378]; Bajaj 1998 [379]; Tomkins 1998 [380]; Al-Chalabi 1999 [381]; Gros-Louis 2004 [382]; Ackerley 2004 [383]; Millecamps 2006 [384]; De Vos 2008 [385]; Oberstadt 2018 [386]; Hergesheimer 2019, 2020 [387, 388]; Volkening 2009 [389]; Prasad 2019 [390]; Alami 2014 [391]; Smith 2014 [392]; Guillaud 2020 [316]; Loeffler 2020 [393]);
- Nephrogenesis, in which defective p75 is implicated (Fédou 2020 [394]);
- Genetic neurogenerative disease, *e.g.* Charcot-Marie-Tooth (CMT) disease (Brownlees 2002 [395]; Jordanova a, b 2003 [396, 397]; Züchner and Vance 2006 a, b [398, 399]; Lancaster 2018 [400]);
- Hereditary spastic paraplegia (Roll-Mecak and Vale 2008 [401]; Dion 2009 [402]; Shribman 2019 [403]; Guillaud 2020 [316]); and
- HIV, human immunodeficiency virus. The role of defective axonal transport was investigated by Berth *et al.*. They do not elucidate the mechanism(s) of fast retrograde axonal transport, *e.g.,* "membrane vis-à-vis cytoskeletal," just that fast retrograde axonal transport was inhibited (Berth 2016 [404]).

4.5 Summary. In summary, this review is an historical narrative of the published literature relating to the selective binding (Section 1), endocytosis (Section 2), and axonal transport (Section 3) of NGF. In reality, these processes are continuous, but are considered here as distinct only for organizational purposes. The key finding of this review is that the historical science not only explains nonclinical results for 800-rhNGF, but importantly, also supports the time criteria for clinical workflow as expressed by practicing oncologic urologic surgeons.

Nerve Growth Factor was discovered 70 years ago, and is in the family of neurotrophins (Appendix H). All neurotrophins and NGF are naturally occurring. Multiple sites endogenously produce NGF in development and in maturity. Cells of the immune system that express TrkA have been identified (Minnone 2017b, Bracci-Laudiero 2003, Prencipe 2014 [61, 405, 406]). When given exogenously, it is definitive that NGF binds selectively to the high affinity TrkA receptor and/or the low affinity, pan-neurotrophin p75 receptor (Section 1). There is also evidence that NGF can be modified to bind, endocytose, and transport other receptors or other molecules.

Finally, it is definitive that retrograde and anterograde axonal transport co-occur. Table C.3 considers all the original studies referred to in Ure and Campenot, 1997 (Ure and Campenot 1997 [23]), and concludes that:

(i) Published rates of axonal transport are best regarded as benchmarks, but should not be considered as precise. This conclusion is due to differences in bioassays, nerve groups in which studies were conducted, and the associated rates of slow and fast retrograde and anterograde transport; and

(ii) *In vitro* experiments roughly confirm what is reported for *in vivo* studies. This provides some measure of credibility to clinical researchers who reference or extrapolate *in vitro* findings to clinical results.

ACKNOWLEDGMENTS

We wish to acknowledge the outstanding contributions made by the following individuals to the science of NGF through their leadership, scientific insights, clinical use, and commercialization of findings:

Contributions to leadership

Stephen B. Kahl PhD, for his inspiration and expertise, and his scientific leadership in bioconjugation chemistry and product development as the Chief Scientific Officer of Manzanita Pharmaceuticals, Inc., Prior to his retirement at UCSF, he is now Emeritus, Vice Chair, Department of Pharmaceutical Chemistry, The University of California, San Francisco; and

Louis Eugene ("Gene") Burton PhD, for his insight into the production of rhNGF, his experience in contributing to the future Chemistry, Manufacturing and Controls (CMC) section of an Investigational New Drug (IND) application, his contributions to developing our own internal source of rhNGF, and his interpretation of analytics for 800-rhNGF and unmodified rhNGF.

Contributions to science

- *Robert B. Campenot PhD (ret., University of Alberta), for his contributions to a body of work in a career devoted to neurobiology, including insights, review of, and comments on this paper;*
- *As consultant to Manzanita Pharmaceuticals, currently at SpectronRx (Indianapolis, IN), Gordon Craig Hill PhD for reducing all of the prototype dye- and drug-neurotrophin conjugates to practice;*
- *At the University of California, Davis, Dr. Pamela J. Lein, Chair and Professor, Neurotoxicology, School of Veterinary Medicine, and Donald Bruun, Lab Manager to the Lein lab. Mr. Bruun conducted all bioassays of 800-rhNGF in superior cervical ganglia (SCGs); and*
- *At Stanford University, Ben Barres MD PhD (1954-2017), formerly Professor and Chair, Neurobiology, Stanford School of Medicine. BenLight is named 800-rhBDNF in Dr. Barres' honor, for his contributions to our studies using 800-rhBDNF and a drug conjugate, fluocinolone acetonide (FA-rhBDNF) in the optic nerve of naïve rats. Also then at Stanford University, we wish to thank Kevin Guttenplan PhD, formerly of the Barres lab, who conducted the in vitro studies of 800-rhBDNF and FA-rhBDNF (Kahl et al., 2019).*

Contributions to clinical use

- *Two of Manzanita's advising MDs, one of whom is an Observer to the Manzanita Board of Directors, and one of whom is an investor-Board member. They both conducted due diligence with the six urologic cancer surgeons who offered their time and detailed thoughts as to surgical workflow, clinical utility, etc.*

Contributions to commercialization

- *All eleven Manzanita investors, five of whom also serve as Manzanita Board members;*
- *The Observers to the Board of Directors of Manzanita Pharmaceuticals, Inc.;*
- *The regulatory affairs firm of SciLucent, Inc., who exceeded our expectations in preparing the "full package" preliminary Investigational New Drug (PIND) questions and briefing to the FDA; and*
- *The FDA Division of Imaging and Radiation Medicine (DIRM), who in their 2021 preliminary Investigational New Drug (PIND) response not only confirmed "structural delineation" as a valid regulatory path, but also noted that "[the c]avernous nerves are generally considered [to be] post-ganglionic parasympathetic nerves arising from the cell bodies in the inferior hypogastric plexus. It is likely [that] the lesser and greater cavernous nerves are identified."*

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

APPENDICES

Appendix A: abbreviations

Although we attempted to maintain consistent nomenclature in this review, those reading the literature may find alternate terms for similar concepts. That terms would evolve over a 70-year history is not surprising.

800	commercial dye that fluoresces in the Near InfraRed (NIR) region
Anterograde	axonal transport from the neuronal cell body (proximal end) to the peripheral end (distal end). Also, orthograde.
BDNF	brain-derived neurotrophic factor
Bioconjugated	conjugation in which the high-affinity TrkA binding sites of a neurotrophin, *e.g.,* Nerve Growth Factor are left intact. This may be via direct attachment of a fluorescent dye to NGF (no linker) or using a heterobifunctional crosslinker for drug-NGF conjugates.
Endocytosis	Process by which NGF-receptor complex is absorbed into a cell, including a neuron. Also, internalization.
HMW	High Molecular Weight, *e.g.,* 7S NGF
HRP	horseradish peroxidase, a neurotracer
IND	Investigational New Drug application to the FDA
ICG	Indocyanine green, a fluorescent dye, first approved by the FDA in the 1950s, still in clinical use.
Internalization	Process by which NGF-receptor complex is absorbed into a cell, including a neuron. Also, endocytosis.
^{125}I-NGF	Iodinated form of NGF
K_d	Concentration for half-maximal saturation, M
kDA	kilodalton, *e.g.* NGF is a 26.3 kDA protein
neuronal cell body	Metabolic center of neuron. Also, soma.
NGF	Nerve Growth Factor
NHP	non-human primate
NIR	Near InfraRed, light outside the spectrum visible to the eye
nm	nanometer, *e.g.,* the 800 NIR range of a fluorescent dye
open	open radical prostatectomy, where the surgical procedure to remove the prostate is performed without laparoscopy
OR	Operating Room
p75	low affinity, pan-neurotrophin co-receptor with Trk receptors
peri-prostatic	surrounding the prostate organ intraoperatively within the surgical incision
PIND	preliminary Investigational New Drug application to the FDA
PK	pharmacokinetics
rhBDNF	recombinant human brain-derived neurotrophic factor
rhNGF	recombinant human Nerve Growth Factor
RP	radical prostatectomy surgical procedure to remove the prostate, here due to cancer
RARP	robot-assisted radical prostatectomy, where the surgical procedure to remove a cancerous prostate is performed with the assistance of a robot in a laparoscopic procedure
Retrograde axonal transport	from the periphery (distal ends) to the neuronal cell body (proximal end); may be TrkA, p75 and/or TrkA+p75 combination mediated
Surface-bound	One possible path of internalization (endocytosis), wherein the dye is observed as a thin line, retrograde axonal transport on the lumen, on the outside of microtubules
Trk	tropomyosin kinase, a class of receptors expressed at the distal (nerve endings) ends of peripheral nerves
TrkA	tropomyosin kinase A receptor

Appendix B: topics not covered

This review does not include, nor do we plan to further review the literature for:

- Clinical studies of NGF or any other neurotrophin;
- Effects when p75 is combined with TrkA;
- The mechanisms for selective binding, their overlap and/or timing of retrograde axonal transport, whether TrkA, p75, and/or their combination;
- The spatial distribution ("map") or quantification of TrkA or p75 receptors, either at or just beneath the surface of the bed of the surgical incision, which incision may be defined at the periphery, nor the effect of applying an NGF-binding conjugate to an anatomical compartment, which compartment would contribute to localization, *i.e.* quantify the effects of receptor-binding and compartmentalization to localizing effects;
- The rate of transport that is p75 mediated, and/or the transition between p75 and TrkA mediated transport (Ure and Campenot 1997a [23]) (i) the efficiency with which NGF is phosphorylated, which may differ at the time of the initial application or later, when 'steady state' has been reached; and/or (ii) the rate at which NGF accumulates in the neuronal cell body prior to degradation (average half-life of 6h) and turns around as anterograde transport;
- Determinants of mechanisms by which retrograde axonal transport cargo is moved in vesicles (membrane-bound), and/or which cargo moves without membranes, not in vesicles (Brown 2003 [191]);
- Consideration of the maturity of TrkA and/or p75 receptors *in vivo;*
- NGF mechanisms and/or functions related to endocytosis, *i.e.,* "signaling" (phosphorylation);
- Pre-operative imaging for cancer, *i.e.,* for perineural invasion. All of our intended surgical cancer procedures involve healthy nerves, a feature confirmed pre-operatively via imaging of the surgical patient;
- A punctate pattern, a characteristic that is typical of staining, can be observed in retrograde axonal transport (Vallee and Bloom 1991; Dillman 1996b; Lalli and Schiavo 2002; Motil 2006; Liu 2007; Perlson 2009; Liu 2012; Smith 2012; Brown 2013 book chapter; Yang 2017 [26, 154, 318, 329, 407-412]);
- Consideration of the relative compartmentalization of 800-rhNGF in the interstitial application to the peri-prostatic space, whether due to surgical incision and or selective TrkA and/or p75 receptor binding;
- Consideration of factors that may be involved in retrograde axonal transport, for example, comparison of bioassays to species and age used in toxicology studies to clinical application, *e.g.*, length of axon, differences in animal models, differences before and after 'steady state' is reached, and/or differences in time to turnaround (retrograde to anterograde);
- "Density" (spatial distribution) of TrkA or p75 receptors exposed either at or near at-risk nerves; and
- Feasibility of using 800-rhNGF or its motor neuron analog 800-rhBDNF as a possible retrograde and/or anterograde tracer.

APPENDIX C: fast and slow rates of retrograde axonal transport of Nerve Growth Factor

C.1 Original investigations (basic research, not disease-related) of rates of axonal transport seem to end in 1997. Axonal transport occurs with multiple proteins, not just NGF, for example, with horseradish peroxidase (HRP), an established neuro tracer. In 1997, Senger and Campenot summarized the evidence of original findings of slow retrograde axonal transport (Senger and Campenot 1997 [10]):

> "Our previous observations estimate the velocity of retrograde transport in compartmented cultures at 10–20 mm/h (Ure and Campenot, 1997 [23]). This is significantly higher than the 2–3 mm/h velocity of NGF retrograde transport reported for adult rat sympathetic axons *in vivo* (Hendry 1974 a,b [32, 164]; Johnson 1978a [76]), but it is within the reasonable biological range since sensory neurons *in vivo* have been reported to transport NGF at 7–13 mm/h (Stöckel 1975b [294]; Yip and Johnson 1986 [295]), and sympathetic axons of the sciatic nerve have been reported to transport dopamine b-hydroxylase at 12 mm/h" (Brimijoin and Helland 1976 [33]).

Brady *et al.* studied fast retrograde axonal transport. He published several basic research papers of original findings and several book chapters (Brady 1982; Brady 1984; Brady 1985 a, b; Brady 1991, Brady 1993 [27-29, 296-298]; Grafstein and Forman, 1980 [274]). In each book chapter (review), Brady restated the various rates and components of axonal transport. This means that most of the work on fast retrograde axonal transport was done by the 1980s.

C.2 Axonal transport: detailed analysis. After 1997, the studies of retrograde axonal transport shifted to disease and mechanism: understanding cargo payload(s), elucidating molecular motors, and signaling, with an increasing emphasis on disease pathology. This analysis focuses on NGF, but does not exclude non-NGF studies;

- The Tables below consider previous reports (finding few), but does not analyze those reports, *i.e.*, no attempt is made to 'harmonize' or normalize findings, or to develop a model;
- Table C.3 lists only original investigations. There were few, almost no new findings in fast retrograde axonal transport since the Brady 1984 summary was published. The main subsequent investigation is by Dillman in his 1996b publication. These investigators describe molecular motors in the slow component of fast anterograde axonal transport ("Slow Component a" or SCa, or "Slow Component b" or SCb) (Dillman 1996b [318]); and
- Note that mechanism is linked to velocity: " … retrograde axonal transport is mechanistically similar to fast anterograde transport except that the immediate source of retrograde transport vesicles is the endocytic pathway rather than the Golgi" (Campenot 2003 [291]).

C.3 Axonal transport: early studies. The review of axonal transport began in 1971 with non-NGF studies of axoplasmic transport, *i.e.,* norepinephrine (Geffen and Livett 1971 [413]; Ochs 1971 [414]). Then in 1972, LaVail and LaVail used HRP to report retrograde axonal transport of 3 mm/h (LaVail and LaVail 1972 [276]) after injecting HRP into the "optic tectum" (the "optic tectum" is now termed "superior colliculus").

The rate reported in the LaVail study was reproduced in a subsequent publication by Hendry *et al.,* 1974a: "…. [the] calculated rate of 2.5 mm/h is similar to the 3 mm/h reported for the transport of [HRP in LaVail and LaVail 1972] from the chick optic tectum to the retinal ganglion cells." In that 1974a report, Hendry *et al.* found the rate of retrograde axonal transport depends on a colchicine-sensitive mechanism, as does the orthograde (anterograde) rapid axonal transport" (Hendry 1974a [32]). Also in 1972, Banks and Mayor reported on rates of axonal transport " … along the axon at a slow rate (about 1 mm/day) whereas others travel at much higher rates (50-240 mm/day)" (Banks and Mayor 1972 [415]).

C.4 Axonal transport: early studies in optic nerve, now understood to be a motor nerve group. The optic nerve and Retinal Ganglion Cells (RGCs) are now considered to be a motor neuron nerve group in the Central Nervous System (CNS). In 1982, BDNF was discovered (Barde 1982 [92]). By 1988, it was known that RGCs express TrkB receptors (Barres 1988 [135]). Studies that calculated the velocities of retrograde axonal transport were conducted in optic nerve in chick, but later studies show that TrkA expression can vary depending on which part of a nerve structure is investigated (Lindqvist 2010 [416]). For example, it is now understood that the cornea and anterior retina are innervated by TrkA receptors (Carmignoto 1989 [417]; Frade 1996 [418], Frade 1999 [419]; Lambiase 1998 [420]; Lambiase 2000 [421]), but that RGCs do not express TrkA (Lindqvist 2010 [416]).

C.5 Axonal transport: Brady 1991 summary. By 1985 (Table C.1; Brady 1985b [29]), enough studies had been conducted of NGF and other moieties, that rates of fast and slow axonal transport (anterograde) could be summarized.

Table C.1 Major Rate Components of Axonal Transport (Brady 1991 [298])

Rate Component	Velocity (mm/Day)	Properties and Composition
Faxt axonal transport		Membrane-bounded organelles
Fast anterograde	200–400	Tubulovesicular structures, synaptic vesicles, membrane-associated proteins, neuropeptides, neurotransmitters and associated enzymes
Fast mitochondria	50–100	Mitochondria, associated enzymes and lipids
Fast retrograde	100–200	Prelysosomal vesicles, multivesicular bodies, multilamellar bodies, growth factors, recycled proteins from fast anterograde transport
Slow axonal transport		Cytoskeletal and cytoplasmic elements
Slow component b	2–6	Actin, clathrin and associated proteins, spectrin, glycolytic enzymes, and calmodulin
Slow component a	0.1–1.0	Neurofilaments, microtubules, and associated proteins

Axonal transport represents a major form of motility for the neuron. The general properties and composition indicate the nature of material being transported and has led to the "structural hypothesis" (see Brady, 1985a, for review). The rates for these different components do not necessarily reflect the rates of the motors involved. For example, if the microfilaments are stationary for 99% of the time and move at rates comparable to fast axonal transport for the remaining 1%, then average rates would be 2 orders of magnitude lower (i.e., 200 mm/day and 2 mm/day).

These rates reported by Brady in 1991 (Brady 1991 [298]) are repeated in later reviews, in book chapters (Brady 1993 [28]; Stenoien and Brady 1999 [36]). Velocities are expressed in mm/day. Note that the velocity of slow retrograde axonal transport reported by Brady refers to anterograde. This differs significantly from the velocity of slow retrograde axonal transport reported by Ure and Campenot in 1997 (10 – 20 mm/h) (Ure and Campenot 1997 [23]).

The possibly erroneous 1985b Brady reference (Brady 1985b [323]) comes from Elluru *et al.,* 1995 (Elluru was in the Brady group) (Elluru 1995 [312]). Elluru published original results of work done in rat optic nerve. Note that what Brady and Elluru designate as "slow axonal transport" refers to the anterograde direction, not the retrograde direction. No speculation is made for this study done in optic nerve, a motor neuron group, vis-à-vis Ure and Campenot's later results in neonatal Superior Cervical Ganglia rat neurons.

> "In mammalian nerves, fast component (FC) represents the movement of membrane bounded organelles (including synaptic vesicle precursors, mitochondria, lysosomes, and their associated proteins) from the cell body toward the nerve terminal (anterograde direction) and from the nerve terminal toward the cell body (retrograde direction) at a rate of 50-400 mm/d. Slow component a (SCa) and slow component b (SCb) move only in the anterograde direction going from cell body toward the nerve terminal at a rate of 0.2-1 mm/d and 2-8 mm/d, respectively. SCa consists of microtubules and neurofilaments, whereas SCb consists of microfilaments, clathrin, and enzymes of intermediary metabolites …"

In 1986, in a study of multiple, non-NGF proteins, McQuarrie *et al.* found differences in slow components (SCa, SCb) and different nerve groups in different species …. " [the] rate of SCa … is 0.7 mm/d in rabbit optic axons and 0.5 mm/d in guinea pig optic axons (Willard and Hulebak 1977 [422]; Levine and Willard 1980 [423]; McQuarrie 1986 [424]). Table C.2 from McQuarrie *et al.* (Table 2, McQuarrie *et al.,* 1986) is reproduced below.

Table C.2 Characteristics of the slow component (McQuarrie 1986 [424])

	Optic axons		Spinal axons	
	SCa	SCb	SCa	SCb
Rate	0.36 mm/d	2.3 mm/d	1.3 mm/d	3.1 mm/d
Primary labeling	−	+	+	−
Composition				
NFT	+	−	+	−
Tubulin	+	−	+	+
Actin	+/−	+	+	+
Calmodulin	−	+	+	+
Clathrin	−	+	+	+
Fodrin	+	+	+	+

C.6 The case for fast retrograde axonal transport

In 1993, Brady published a book chapter which noted how retrograde and anterograde transport could be fast, at "several microns per second" (Brady 1993 [28] Chap 9), but that " … slow transport conveys cytoskeletal and cytoplasmic proteins and advances at a rate of no more than a few microns per minute."

In 1997, Ure and Campenot (Ure and Campenot 1997 [23]) in a rigorous study, reported on slow retrograde axonal transport, then speculated about rates in immature neurons. They found that:

> "[a] second aspect is the rapid transport velocity for NGF of 10–20 mm/hr. This velocity matches reported retrograde organelle velocities from a variety of axons (Forman 1977 a, b [425, 426]; Smith and Cooper 1981 [321]; Koles 1982 [427]; Breuer 1987 [428]; Abbate 1991 [429]), which suggests that once NGF is loaded onto the transport mechanism it is optimally delivered to cell bodies. The several-fold, higher velocity that we observed, as compared with that in sympathetic neurons *in vivo* (Hendry 1974 a,b [32, 164]; Johnson 1978a [76]), might suggest that the velocity of retrograde transport is faster in immature neurons used for culturing than in adult neurons used in the *in vivo* studies." ….. "It is possible, however, that the slightly lower transport rates that were observed when using the highest NGF concentration (40 ng/ml) might have resulted from increased binding to p75, because several studies indicate that p75 internalizes and/or transports NGF at a low rate, if at all (Le Bivic 1991 [430]; Kahle and Hertel 1992 [431]; Kahle 1994 [432]; Mahadeo 1994 [433]; Curtis 1995 [142]).

Earlier investigators studied the effects of temperature, gender or age (Forman 1977 a,b [425, 426]; Smith and Cooper 1981 [321]). For example, roughly 50% of men undergoing radical prostatectomies are 65, but whether or not the receptors exposed in surgery are the age of the mammal, or newer because they are not peripheral, or have been exposed to a constant body temperature, is unknown.

C.7 The p75 receptors: pan-neurotrophin, but multi-faceted. The low-affinity p75 receptor was discovered in 1973 (Johnson 1986 [109]; Radeke 1987 [110]). Discovery of p75 was prior to the elucidation in 1985 of the TrkA receptor (Hosang and Shooter 1985 [40]). Discovery of p75 was also made prior to the understanding that p75 can bind to other neurotrophins as well as NGF (Frade 1999 [419]; Bibel 1999 [434]; Bibel and Barde 2000 [253]; Barker and Shooter 1994 [105]), and prior to the finding that TrkA may be complexed with p75 (He and Garcia 2004 [435]).

It is of critical importance that binding of p75 is differentiated from NGF binding to TrkA:

- It is known that p75's binding to TrkA differs from its binding to other Trks (Ibanez 1992 [101]). For example, Barker *et al.,* 2004 described p75 as "a component of three distinct [components that] allows the Trks to respond to limiting neurotrophin receptor platforms that bind different ligands …. [to] facilitate cell survival … cell death, or growth inhibition" (Barker 2004 [436]).
- When binding to the pro form of NGF, p75 may take apoptotic role (Dechant and Barde 2002 [437]; Barker 2004 [436]; Ibanez and Simi 2012 [438]; Hempstead 2014 [62]; Trouvilliez 2023 [45]). Only the pro-form, not the mature form of NGF, when NGF binds to p75, can be apoptotic (Barker 2004 [436]). The Hempstead group established that proNGF binds with high affinity to p75:

  > "Using a furin-resistant form of proNGF, Hempstead and colleagues (Lee 2001 [439]; Nykjaer 2004 [440]) found that proNGF binds p75NTR with high affinity and is a potent inducer of p75NTR-dependent apoptosis in sympathetic neurons, oligodendrocytes, and in a vascular smooth muscle cell line. These investigators also showed that proNGF does not bind TrkA and suggested that proNGF is an apoptotic ligand that is specific for p75NTR."

As noted above, the proNGF form does not, or poorly binds to TrkA, but mature NGF competes with TrkA and p75 receptors (Lee 2001 [439]; Nykjaer 2004 [440]; Kliemannel 2007 [18]; Hempstead 2014 [62]). As a result, the pro-apoptotic property of the proNGF may have led to the characterization of TrkA as a "proto-oncogene." Importantly, the 800-rhNGF conjugate uses only the mature form – which does bind to TrkA.

Unlike the literature documenting the location of the high affinity TrkA receptors at the distal ends, to our knowledge, there are only a handful of studies that implicate the location of p75 receptors as also being at the distal ends. Due to the number of studies that appear to mix TrkA with p75 receptor binding – including in PC12 cells - when co-receptor binding occurs, we only assume that p75 is located with Trk receptors. This places p75 receptors at the distal ends of neurons (Toma 1997 [66]). However, with the exception of disease studies that include distribution studies, there are fewer publications of the tissues that endogenously express the pan-neurotrophic receptor (Barrett 2005 [441]; Underwood and Coulson 2008 [442]; Pham 2019 [443]).

C.8 Axonal transport: reports of slow retrograde axonal transport. In Table C.3 below, we consider original investigations and compare the findings of early to later reports, in fast and slow retrograde axonal transport. There appear to be no significant updates after 1997, with the sole exception of Dillman *et al.,* 1996. The last rigorous study of axonal transport we identified was for slow retrograde axonal transport, reported as 10-20 mm/h by Ure and Campenot (Ure and Campenot 1997 [23]) in an *in vitro* system of sympathetic neonatal rat neurons. These authors found that rate to be within a "reasonable biologic range" (Senger and Campenot 1997 [10]). Subsequent findings report results of investigations with non-NGF (Melemedjian 2014 [444]; Mar 2014 [445]).

Table C.3. Rates of retrograde and anterograde (orthograde) axonal transport

		Model		References & notes
Fast retrograde axonal transport (200-400 mm/d). Includes NGF, non-NGF studies				
50-240 mm/d		(unknown; sources and references not given)		Banks and Mayor 1972 [415]
Unknown		*In vivo*, extruded axoplasm, **squid**		Brady 1982 [296]
± 432 mm/d small particles Average fast and slow: 216 mm/d		*In vivo*, extruded axoplasm, **squid**		Allen 1982 [34]
10-20 mm/h (retrograde 0.91 µm/sec; 0.93 anterograde µm/sec)		*In vivo, Xenopus laevis,* **African clawed frog**		Koles 1982 [427]
(unknown)		*In vivo*, extruded axoplasm, **squid**		Leopold 1990 [302]
		REVIEW, includes early studies of molecular motors, new techniques. See multiple references		Brady 1991 [298]
		REVIEW, book chapter 9		Brady 1993 [28]
(kinesin organelles)		*In vivo*, retina, optic nerve, **rat**		Elluru 1995 [312]
		REVIEW, considers protein synthesis in long axons		Campenot and Eng 2000 [290]
CREB, IL-6 plasticity		*In vivo*, DRG, **mouse**		Melemedjian 2014 [444]
Dynein dynactin-50, β amyloid precursor protein (βAPP)		*In vivo*, brain **human** (Alzheimer's)		Aboud 2015 [304]
p75-AF647 conjugate		*In vivo*, sciatic nerve, in life, in anesthetized **mouse**		Gibbs 2015 [292]
p75-AF647 conjugate		**METHODS REVIEW.** *In vivo*, sciatic nerve, in life anesthetized **mouse**		Sleigh 2016 [293]
CREB, IL-6 plasticity		*In vivo*, DRG, **mouse**		Moy 2018 [446]
Slow retrograde axonal transport (10-20 mm/h)				
	in vitro	*in vivo*		
NGF a, NGF b	(a) - 2.5 mm/h (b) about 2.2-2.5 mm/h		*In vitro*, a – anterior intra-ocular injections, **mouse**; b – anterior intra-ocular injections, **rat, mouse**	Hendry 1974a, b [32, 164]
NGF a, NGF b		(a) 2.5 mm/h (b) 13 mm/h	*In vivo*, a – anterior intra-ocular injections in superior cervical ganglia and submaxillary gland, **mouse**; b – injected forepaw, **rat**	Stoeckel a, b 1975 [88, 294]
dopamine-β-hydroxylase	12mm/h		*In vitro*, sciatic nerve, **rabbit**	Brimijoin and Helland 1976 [33]
		3 mm/h	*In vivo*, a – anterior intra-ocular injections, **rat, hamster**	Johnson 1978a [76]
NGF	3 mm/h		*In vitro,* compartmented cultures, superior cervical ganglia, **neonatal rat**	Claude 1982b [283]
non-NGF transported proteins			*In vivo*, optic nerve, **rat;** spinal nerves, **rat**	McQuarrie 1986 [424]
NGF, HRP	7 mm/h		*In vivo*, crushed sciatic nerve into DRG, **rat**	Yip and Johnson 1986 [295]

	(b) 2-8 mm/d SCb retrograde; SCa 0.2-1 mm/d		Optic nerve, RGCs, **rat**	Dillman 1996 b [318]
NGF			*In vivo*, in sciatic nerve and hippocampus (fimbria-fornix), **rat**	Johnson 1987 [77]
NGF	10-20 mm/h		*In vitro*, in compartmented cultures, **rat**	Ure and Campenot 1997 [23]
NGF, other NTs	10-20 mm/h		*In vitro,* in compartmented cultures, **rat**	Senger and Campenot 1997 [10]
Brefeldin A			*In vitro,* in compartmented cultures, **rat**	Campenot 2003 [291]
Non-NGF: anterograde fast & slow axonal transport				
(unknown)	1 mm/h ("centrifugal" anterograde)		(source not given)	Banks and Mayor 1972 [415]
HRP	3 mm/h		*In vitro*, optic nerve (optic tectum ~ superior colliculus May 2014 [447]), **chick**	LaVail and LaVail 1972 [276]
MAP 1 (microtubule-associated protein 1)			*In vitro,* in multiple mammalian cells	Bloom 1984 [299]
Novel ATPase			*In vitro*, brain, **chick**	Brady 1985a [297]
monocrystalline iron oxide nano-compound (MION) imaging agents	5 mm/d		*In vivo*, sciatic nerve, **rat**	Enochs 1993 [448]
p75 and TrkB (NGF as control)		(not given)	*In vivo*, optic nerve, **chick**	von Bartheld 1996 [178]
MAP 1C			(a) *in vitro;* (b) *in vivo*, in brain, **calf**	Paschal 1987a [300]; Paschal 1987b [301]
PC12 cell-based studies				
NGF			*In vitro*, PC12 cells	Yankner and Shooter 1980 [305]
NGF			*In vitro,* PC12 cells	Schechter and Bothwell 1981 [307]
NGF			*In vitro*, PC12 cells	Hosang and Shooter 1985 [40]
NGF, HSV-1 p75			*In vitro*, PC12 cells	Battleman 1993 [168]
NGF, other NTs			*In vitro,* in PC12 cells	Treanor 1995 [30]
p75			*In vitro*, PC12 cells; *in vivo*, retina, optic nerve, **chicken eggs**	Butowt and von Bartheld 2009 [31]

C.9 Axonal transport in PC12 cell lines: why studies in PC12 cells are included in Table C.3. Studies *in vitro* in PC12 cells (rat pheo-chromocytoma = PC12 cells; Greene and Tischler 1976 [449]; Greene and Rein 1977 [450]) were included in this review because these cells do bind to TrkA, and possibly also to p75. However, because the PC12 cell line does not recapitulate neuroanatomy, PC12 cells cannot assess transport, only NGF receptor binding and neurite outgrowth.

C.10 Topics not studied here, but will be discussed in future nonclinical studies. We do not plan to undertake future analyses of either functional (delivery) or pathological axonal transport:

- Comparison rates of retrograde and anterograde axonal transport to differences in TrkA and/or p75 and/or their combination in receptor binding (Ultsch 1999 [98]; He and Garcia 2004 [435]; Wehrman 2007 [451]);
- " … [s]ince the domains that were implicated in mediating TrkA/p75 interactions were not included in the original NGF/TrkA-D5 crystal structure it has not been possible to evaluate a structural model of the complete receptor" (Gong 2008 [452]; review in Guo 2020 [368]); differences in length or type of axon (McQuarrie 1986 [424]); differences in animal models for *in vitro* assays or *in vivo* measurements; and/or differences in time to turnaround (retrograde to anterograde);
- Confirmation mechanistically that NGF binds first to p75, then is transported either via fast axonal retrograde transport in a membrane, or as a p75-TrkA-NGF complex in a cytoskeletal complex. A scenario with both mechanisms is consistent with the literature. " … After a short lag period, high-affinity binding also appears and increases slowly."
- Conversion of p75 to TrkA, or under what circumstances (Landreth and Shooter 1980 [306]); and

> " …. NGF binds first to receptors of low affinity and that the binding induces a conversion of a proportion of the receptors to a higher affinity state. It is also consistent with a model in which the change in affinity is due either to conformational changes in the receptor or to interaction of the occupied receptor with other receptors or with effector proteins in cell plasma membrane" (Landreth and Shooter 1980 [306]).

- Confirmation of the diametric size of illuminated neurons, and differences in species. In 1986, Yip speculated that diameter size could be important in considering transport: " …. small diameter neurons retrogradely transport and turnover NGF faster than larger diameter neurons …" (Yip 1986 [295]). Others may investigate species, nerve site and length and diameter size. The mechanism of size of neuron (diameter, but possibly also axonal length) may not be important. In reports that the NGF-TrkA and/or NGF-receptor complex is retrogradely transported on axons when it is 'surface-bound,' or in the lumina, on the outside of microtubules, which are cytoskeletal structures (Peters 1968 [20]; Rodriquez Echandia 1968 [21]; Burton P.R. 1984 [22]; Ure and Campenot 1997 [23]; Garvalov 2006 [24]), findings do not report on the efficiency or analyze the varying shapes and sizes of what is transported.

Appendix D: TrkA is described in early studies as a proto-oncogene

800-rhNGF uses the mature form, not the pro-form of NGF (Lee 2001 [439]; Nykjaer 2004 [440]; Kliemannel 2007 [18]; Hempstead 2014 [62]; Masoudi 2009; Iouannou and Fahnestock 2017 [16, 453]). The binding of the mature form to TrkA is significant because it is the pro-apoptotic property of proNGF binding to p75 that may have led to the characterization of TrkA as a "proto-oncogene." Briefly, the genetic background to cancer appears rarely ~ 1% of the time, and appears to involve "gene fusions" – such "internal rearrangements" do not argue against an NGF-receptor binding mechanism.

Hempstead *et al.* conducted a series of experiments in 1991, concluding that both receptors – "two different low-affinity receptors" were necessary for *binding*, but they did not study retrograde axonal transport (Hempstead 1991 [94]). Of interest, the TrkA receptor was then referred to as an "proto-oncogene" or "oncogene" (Kaplan 1991 b, a [228, 231]; Klein 1991a [81]; Lamballe 1991b [454]). From Lamballe 1991:

> "To date more than [25] different oncogenes have been identified in human neoplasias. One of these oncogenes is trk, a transforming gene originally isolated from a colon carcinoma biopsy by using gene transfer assays. This oncogene encodes a chimeric molecule that contains the 221 amino terminal residues of a non-muscle tropomyosin followed by the transmembrane and cytoplasmic domains of the trk proto-oncogene product, a tyrosine protein kinase receptor. trk oncogenes have also been identified in a significant fraction of thyroid papillary carcinomas. Some of these trk oncogenes contain sequences derived from genes other than tropomyosin. One such gene is tpr, a gene first identified as a component of the human met oncogene. The trk proto-oncogene, non-muscle tropomyosin and tpr map in the long arm of chromosome 1. Therefore, trk oncogenes are likely to result from internal rearrangements or from unequal cross-overs between two chromosome 1s. Recent studies have demonstrated that the product of the trk proto-oncogene, gp140trk, is the functional receptor for nerve growth factor (NGF) …." (Lamballe 1991b [454])

Luberg *et al*.'s report in 2015 (Luberg 2015 [14]) elucidated the "presence of multiple (TrkA) isoforms." This suggested to us that healthy TrkA isoforms may not be oncogenic at all. We are aware of no evidence that single, microdose rhNGF binding to TrkA activates or accelerates cancer (Lubert 2015; Kummar 2018; Amatu 2019; Milione 2017; Jang 2009; Jang 2007; Zheng 2016 [14, 455-460].

Rarely, cancer involves roughly 80 Trk gene fusions (Amatu 2019 [456]). These rare cancers involve up to 12 tumor types (Milione 2017 [457]; Kummar 2018 [455]). Kummar *et al.* reported that "[o]ncogenic somatic chromosomal rearrangements involving the NTRK1 (TrkA), NTRK2 (TrkB), or NTRK3 (TrkC) genes (NTRK gene fusions) occur in up to 1% of all solid tumors, and have been reported across a wide range of tumor types" (Kummar 2018 [455]; Jiang 2021 [75]). These cancers may be treated with larotrectinib (Viktravi® and TRK inhibitor, LOXO-195), a tyrosine kinase inhibitor of TrkA, TrkB and TrkC. However, approved treatment – and possibly also the Trk gene fusion hypothesis – has been questioned in the EU, where larotrectinib has not proved to be efficacious (Trouvilliez 2023 [45]).

Larotrectinib was discovered by Array BioPharma [461, 462], then subsequently licensed in 2015 to Loxo Oncology and Bayer Pharmaceuticals, Inc. Loxo was acquired by Lilly in 2019. Larotrectinib is sold jointly by Loxo/Lilly and Bayer. Larotrectinib is sold at $400,000 per year, "[b]ut along with [the $400,000 price] came a money-back guarantee: a full refund to payers when patients don't respond within 90 days" (Weintraub Fierce Pharma 2019 [463].

Larotrectinib was approved by the FDA in 2018 (FDA 2018 [464]). It is a systemic medication, given at 100 mg orally twice daily for adults, “until disease progression or until unacceptable toxicity”) (Bayer HealthCare 2018 [465]. In a 30-day course, this would equal 6,000 mg (in rat, dose for 800-rhNGF was established at 1 mg/ml, or roughly 50 µg).

Appendix E: next steps, including preliminary benchmarks of component safety

E.1 Why we are cautiously optimistic about the safety and tolerability of 800-rhNGF: because its individual components are known to be safe at higher doses

The FDA requires us to rigorously test the 800-rhNGF conjugate – not the components - in GLP toxicology and safety pharmacology studies. However, both unmodified components have an established clinical profile.

- Repeat dose unmodified rhNGF (20 µg/ml 6X QD for 8 weeks, total 6,720 µg/ml) as eye drops were approved by the EMA in 2017 and by the FDA in 2018 (FDA 2018 [466]) to treat neurotrophic keratitis (Lambiase 2000; Roberti 2014; Bonini 2018a; Bonini 2018b [421, 467-471]).
- We selected this Near InfraRed (NIR) 800 dye because (i) it is available to us without a license as a non-GMP research material; (ii) its chemistry is amendable to conjugation, but the structure of ICG is not; (iii) it is similar to other fluoresceins used clinically that have very low toxicity and are clinically safe (Munch 2020; Koch 2017; Lamberts 2017; Gao 2018; Nishio 2020 [472-476]) (iv) it is similar to the LI-COR IRDye 800CW®; (v) as reported in a US patent 10,405,753 B2, when tested in exhaustive nonclinical studies, it obtained no mortality and no toxicity at 80X systemic dose in dog, and 160X systemic dose in rat (USPTO Sorger 2019 [477]). “Formula three” is the dye reported in that issued patent as used in 800-rhNGF; and (vi) due to its optical properties, the dye component can be low (in 5 µL, 8% of total volume; by MW 1050, rhNGF monomer 13.5 kDA); and
- After careful comparison of chemical structures, we are confident that this “formula three” NIR dye is the same dye as that used by Intuitive Surgical in 2021 in a completed clinical Phase 2 study NCT03937505 (n=94) (clinicaltrials.gov 2023 [478]). In a prior first-in-human (women) 2017 study NCT03006237 (n=24), also to test safety and tolerability as well as pharmacokinetics, the same interventional agent IS-001 was administered during surgery, single dose, intravenously (IV) for women undergoing a hysterectomy at 10mg, 20mg or 40 mg. “At 10 min postinjection, the 40-mg dose-cohort showed the highest fluorescence intensity ….” (Farnam 2019 [479]).

E.2 Other safety considerations

Nonclinical results will be published separately.

The regulatory path for developing 800-rhNGF as a ‘surgical guidance tool’ is established. This FDA path is the same as that followed for approval of ICG. Note that surgical guidance is governed by “structural delineation,” *i.e.,* we do not seek to reduce complications (“clinical outcomes,” a separate regulatory path) of any surgical therapy for an oncologic cancer.

Appendix F: suggested further reading

The following references are suggested for further reading (by year):

- "Physiology of Nerve Growth Factor" (Thoenen and Barde 1980 [282]);
- "trkB encodes a functional receptor for brain-derived neurotrophic factor and neurotrophin-3 but not nerve growth factor" (Squinto 1991 [85]);
- "The Trk family of tyrosine kinases: receptors for NGF-related neurotrophins" (Parada 1992 [480]);
- "Cell biology of neuronal endocytosis" (Parton and Dotti 1993 [39]);
- "Rapid retrograde tyrosine phosphorylation of trkA and other proteins in rat sympathetic neurons in compartmented cultures" (Senger and Campenot 1997 [10]);
- "Retrograde transport and steady-state distribution of ^{125}I-nerve growth factor in rat sympathetic neurons in compartmented cultures" (Ure and Campenot 1997 [23]);
- "Receptor binding, internalization, and retrograde transport of neurotrophic factors" (Neet and Campenot 2001 [8]);
- "Neurotrophins: roles in neuronal development and function" (Huang and Reichardt 2001 [157]); "Trk receptors: roles in neuronal signal transduction" (Huang and Reichardt 2003 [255]);
- "Retrograde support of neuronal survival without retrograde transport of nerve growth factor" (MacInnis and Campenot 2002 [241]);
- "Neurotrophin-regulated signalling pathways" (Reichardt 2006 [481]);
- "Retrograde transport of neurotrophins: fact and function" (Campenot and MacInnis 2004 [365];
- "Structural and mechanistic insights into nerve growth factor interactions with the TrkA and p75 receptors" (Wehrman 2007 [451]);
- "Endocytosis and signalling: intertwining molecular networks" (Sorkin 2009 [482]);
- "Lysosome and Endosome Organization and Transport in Neurons," book chapter by Gindhart and Weber (Gindhart and Weber 2009 [193]);
- "NGF uptake and retrograde signaling mechanisms in sympathetic neurons in compartmented cultures" (Campenot 2009b [185]);
- "Biogenesis and function of the NGF/TrkA signaling endosome" (Marlin and Li 2015 [195]);
- "Retrograde signaling via axonal transport through signaling endosomes" (Yamashita 2019 [483]); and
- "High-affinity TrkA and p75 neurotrophin receptor complexes: A twisted affair" (Conroy and Coulson 2022 [246].

Appendix G: definition of families of neurotrophins

G.1 "Family" of neurotrophins: narrow and expanded definitions

Nerve Growth Factor was the first of several neurotrophins to be discovered. All neurotrophins, both NGF and other subsequently elucidated neurotrophins such as brain-derived neurotrophic factor (Barde 1982 [92]):

- Are proteins that occur naturally in mammals, including rat, dog, swine, and human;
- Occur first as proneurotrophins, then *e.g.,* NGF in mature form (Fahnestock 2001 [15]; Hempstead 2014 [62]; Ioannou and Fahnestock 2017 [16]; Shekari and Fahnestock 2019 [63]);
- Bind to a specific high-affinity receptor, where high – but not exclusive - affinity is determined by genetic encoding to a tropomyosin kinase (Trk) receptor. For NGF, the high affinity receptor is TrkA;
- After the neurotrophin binds to the Trk receptor, the neurotrophin-Trk complex is internalized;
- The internalized complex is transported retrogradely ("axonal retrograde transport") in either fast and/or slow retrograde, then anterograde (orthograde) axonal transport; and
- In addition to the high-affinity Trk receptor, also bind to the low-affinity, pan-neurotrophin receptor, p75.

The family of neurotrophins includes NGF, BDNF, NT-3, and NT-4 (Barde 1982 [92]; Liebrock 1989 [484]; Jones 1990 [485]). "Family" may be defined more broadly to include other neurotrophin-like proteins that also require two receptors to act.

Since the founding discovery of the first neurotrophic factor, Nerve Growth Factor (NGF) in 1952 by Rita Levi-Montalcini MD PhD (Levi-Montalcini 1952 [1]; Aloe 2004 [5]; Aloe 2012 [6]), the pleiotropic mechanisms of NGF and other proteins in the family of neurotrophins including brain-derived neurotrophic factor, BDNF; neurotrophic factor 3, NT-3 and others like ciliary neurotrophic factor (CTNF) have been elucidated.

Early reports explored how NGF affected metabolism (Levi-Montalcini 1964 [486]; Angeletti 1964 [487]; Levi-Montalcini and Angeletti 1968a [488]; Levi-Montalcini 1968c [489]; Angeletti 1968 [490]; Zanini 1968 [491]). These early reports contributed to later clinical investigations of exogenously administered NGF, via several routes, for numerous neurodegenerative diseases.

The family of neurotrophins includes NGF, BDNF, neurotrophin 3 (NT-3) and NT-4/5. A broader definition of family includes proteins in the transforming growth factor beta (TGF-β) family that includes glial derived neurotrophic factor, GDNF and ciliary neurotrophic factor, CNTF.

G.2 BDNF and TrkB interact similarly to NGF and TrkA

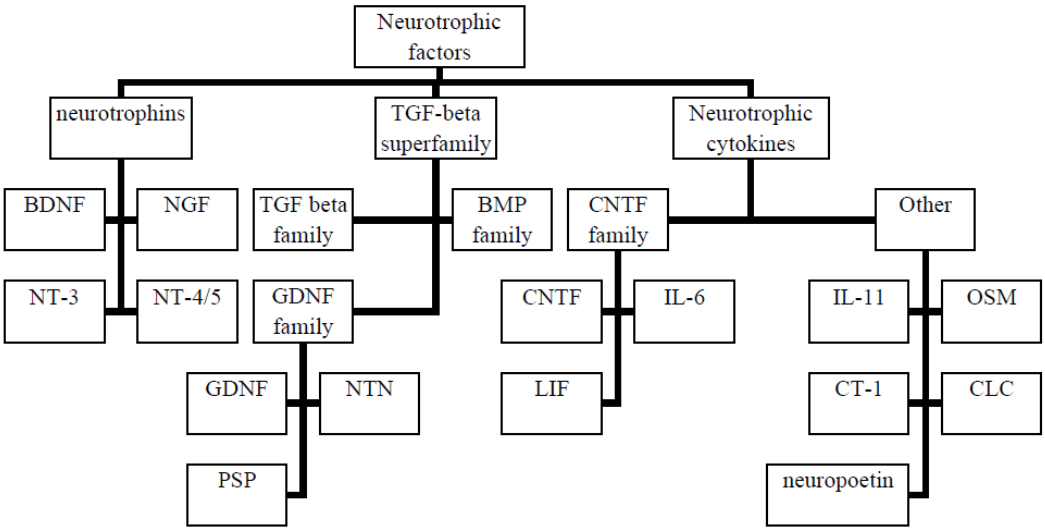
Following the discovery of BDNF in 1982 (Liebrock 1989 [484]; Barde 1982 [92]), and then the TrkB receptor in 1989 (Klein 1989 [79]; Li 2023 [492]), structural considerations (Ultsch 1999 [98]) and mutagenesis data (Ibanez 1993 [493]) suggest that BDNF interacts with TrkB like NGF interacts with TrkA. BDNF is about 55% homologous to NGF, sharing five of 11 conserved lysine residues. BDNF is a dimer of two identical 119 amino acid subunits of MW 28 kDA held together by strong hydrophobic interactions. When BDNF binds to TrkB at distal ends (Klein 1991b; Ultsch 1999; McMahon 1994; Kashiba 1995; Yamamoto 1991; Gibbs 2015; Sleigh 2016 [83, 98, 160, 292, 293, 494-496]), BDNF-TrkB complexes are internalized into signaling endosomes that are moved retrogradely along axons (Neet and Campenot 2001; Watson 1999; Heerssen and Segal 2002; Ahn 2000; Ginty and Segal 2002; Banfield 2001 [8, 233, 262, 497-499]). Like NGF-TrkA, BDNF-TrkB is transported retrogradely to neuronal cell bodies (Watson 1999; Banfield 2001; Robinson 1995 [233, 499, 500]).

G.3 The co-receptor concept expands the "family" concept of neurotrophins

Both GDNF and CNTF have been studied clinically. Both are known to be activated by co-receptors, where one receptor confers selectivity. In principle, we could use GDNF or CNTF instead of NGF or BDNF.

Figure G.1 Source: Figure 1, Kalinowska-Lyszczarz and Losy 2012 [501]

Figure 1. Neurotrophic factor family classification. NGF: nerve growth factor, BDNF: brain-derived neurotrophic factor, NT-3: neurotrophin 3, NT-4/5: neurotrophin 4/5, TGF beta: transforming growth factor beta, GDNF: glial derived neurotrophic factor, BMP: bone morphogenic proteins, NTN: neurturin, PSP: persephin, CNTF: ciliary neurotrophic factor, IL-6: interleukin 6, LIF: leukemia inhibitory factor, IL-11: interleukin 11, OSM: oncostatin M, CT-1: kardiotrophin-1, CLC: cardiothrophin-like cytokine.



- For GDNF, the co-receptors tyrosine kinase RET and the GFR-alpha (GFRα) were discovered in the late 1990s (Barbacid 1995a [502]; Yano 2000 [259]; review, Ibañez 2020 [503]; review, Fudalej 2021, see Table 1 [64]). RET is an abbreviation of 'rearranged during transfection,' as the "DNA sequence of this gene was originally found to be rearranged within a 3T3 fibroblast cell line following its transfection with DNA taken from human lymphoma cells" (Takahashi 1985 [504]). In the GDNF family are GDNF, artemin, neurturin, and persephin; and

- CNTF is often grouped with the interleukin-6 (IL-6) family of cytokines, or neurotrophic cytokines in the CNTF family. For CNTF, there are three co-receptors: CNTF alpha-receptor (CNTFR), the beta-receptor gp130, and leukemia inhibitor factor receptor (LIFR) (Rose-John 2018 [505]).

G.4 Rationale for selecting neurotrophins as neuronal targeting vectors

We selected neurotrophins – rhNGF and rhBDNF - as neuronal targeting vectors because (i) their clinical safety has been shown; (ii) their selectivity for nerve groups – sensory and motor, respectively - is established; (iii) they are off-patent, so all neurotrophins may be considered for biosimilar development; (iv) they act intracellularly, *i.e.,* do not bind to myelin; and (v) our studies, as well as mutagenesis studies, suggest that binding events for intracellular delivery may be physically distinguished from biochemical sites, *i.e.,* their selectivity properties may be harnessed to deliver a range of small molecule drugs. Clinical studies of repeat dose unmodified recombinant human NGF (rhNGF) have shown that rhNGF, at least in an anatomical compartment, is non-immunogenic (Ferrari 2014; Bonini 2018a; 2018b [468, 469, 506]).

The family of neurotrophins includes the soluble peptides NGF, BDNF, neurotrophic factor 3 (NT-3), neurotrophic factor 4/5 (NT-4/5), neurotrophic factor 6 (NT-6) (Barde 1982; Liebrock 1989; Jones 1990 [92, 484, 485]), and neurotrophic factor 7 (Campenot 2004 [365]).

As shown in Figure 1 from Kalinowska-Lyszczarz and Losy (Kalinowska-Lyszczarz and Losy 2012 [501]), both glial-derived neurotrophic factor (GDNF) (Chang 2019 [507]; Skaper 2018 [508]) and ciliary neurotrophic factor (CNTF) (review, Richardson 1994 [509]) may be considered in the neurotrophic family, even though technically they may be considered to be in different subfamilies (Kalinowska-Lyszczarz and Losy 2012 [501]). The crystal structure of both CNTF and GDNF have been elucidated:

- Ciliary neurotrophic factor (CNTF) is a dimer (McDonald 1995 [510]) which binds selectively with three distinct receptor subunits (specific cell surface receptor, CNTF-Rα, permitting the subsequent recruitment of the receptors gp130 and leukemia inhibitory factor beta (LIF-Rβ); and
- Similarly, GDNF is also a dimer (Eigenbrot 1997 [511]). For GDNF, “physiological responses to GDNF require the presence of a novel glycosyl-phosphatidylinositol (GPI)-linked protein (designated GDNFR-α) that is expressed on GDNF-responsive cells and binds GDNF with a high affinity…. GDNF promotes the formation of a physical complex between GDNFR-α and the orphan tyrosine kinase receptor Ret, thereby inducing its tyrosine phosphorylation … GDNF uses a multi-subunit receptor system in which GDNFR-α and Ret function as the ligand-binding and signaling components, respectively” (Treanor 1996 [512]).

Appendix H: axotomization, when nerves are severed and regrow

H.1 Axotomy: definitions

Briefly, we consider the science of axotomization, or what happens when TrkA receptors are lost, for example, when nerves are cut deliberately. For example, this occurs when making an initial surgical incision into skin. We review studies in which ‘nerve endings’ – where TrkA receptors are expressed, to which the NGF component of the dye-NGF conjugate binds – sprout or regrow due the effects of endogenous and/or exogenous NGF binding on TrkA. It is also well known that withdrawal of NGF arrests sprouting.

All surgical incisions – in oncology and non-oncology - deliberately axotomize nerves, concurrently leaving TrkA and possibly p75 receptors in three locations: at the surface of the incisional bed; just below the surface; and fully axotomized (no receptors, both TrkA and p75 receptors are removed) at the surface of the incisions. We expect that surgical incisions, nerve anatomy, and access (“density” or spatial distribution) of TrkA-p75 receptors in that incision will differ from disease to disease, surgery to surgery, and case to case (patients are individualized).

In none of the nonclinical studies to date of 800-rhNGF was any regrowth or sprouting observed.

Axotomization is well defined, but it is important to distinguish between:

- Acute and secondary axotomy (surgical incision is acute; a trauma to a nerve, for example in a nerve crush, occurs relatively slowly over time, this type of axotomy is secondary);
- If a study is *ex vivo*, to consider time when the study is conducted from the time of euthanization. Since the “NGF death signal” for neurodegeneration begins soon after termination (1h), studies in human cadavers

are not feasible (Campenot 1981 [360]; Seeley and Greene 1983 [361]; Campenot 1987 [116]; Martin 1988 [362]; Deckwerth and Johnson 1993 [363]);
- Whether the NGF source is endogenous and/or exogenous NGF, and which form of NGF is used for these studies (proNGF or mature NGF);
- If NGF is given exogenously, whether the dose is systemic or local (for example, two routes – intrastitial and intranasal – do not engage cutaneous TrkA receptors), the dose level, and whether the dose is single or repeat;
- Stage of development, *i.e.* axonal termination in a developing mammal is normal and healthy; and
- Clinical settings where sprouting (axonal regrowth and synaptic reconnection) is encouraged, for example, after spinal cord injury.

H.2 Axotomy: clinical considerations

A single, low dose of a neurotrophin-dye or neurotrophin-drug conjugate given locally should be safe:

- If sprouting does occur, it will be minimal: (i) it may be induced by the dose component of NGF in bioconjugates that use NGF, but probably not the conjugate payload; or (ii) sprouting will be localized to the surgical location where TrkA receptors are axotomized; and
- It would only occur until degeneration due to NGF withdrawal occurs, roughly in 1 da after axotomy or incision (Takaso 2020 [513]).

The risk of acute axotomy accelerating cancer pathology due to the effects of the NGF component in a single, low dose conjugate, in a disease that the surgeon is trying to treat by cutting nerves at the skin, is virtually nil – even after millions of surgical procedures. There are a handful of reports of NGF in prostate cancer, but none appear to study the same nerve groups identified at-risk, the cavernous nerves. For example, in prostate cancer, this group was focused on the contribution of TrkA receptors in skeletal tissue to bone cancer pain. They did not use acute axotomization in their experimental study, but focused on the activity of blocking – not upregulating – NGF activity (Jimenez-Andrade 2010 [514]; Castaneda-Corral 2011 [515]).

It is established that at maturity, endogenous NGF regulates axonal sprouting (Bennett 1998 [516]; Spillane 2012 [517]; Minnone 2017b [61]). Following non-localized, systemic application, “sensory neurons do not sprout significantly after systemic NGF administration” (Levi-Montalcini and Booker, 1960a [518]). That systemic NGF does not induce sprouting in sensory neurons is consistent with later findings that systemic NGF binds to TrkA on circulating immune cells. It is known that TrkA is expressed on several types of immune cells. As noted above, this suggests that any NGF-receptor complex will be degraded rapidly by esterases and proteases in plasma - not transported retrogradely in axons (Torcia 1996 [519]; Bracci-Laudiero 2010, 2002, 1993, 2003 [405, 520-522]; Prencipe 2014 [406]; Minnone 2017b [61]).

First, in sympathetic ganglia (neurons) *in vitro* and *in vivo*, at ‘nerve endings’ - where the TrkA receptors were located, and subsequently identified as being expressed – it was noted that exogenous NGF would exert local control over neurite sprouting (Hendry 1975 [122]; Campenot 1977, 1987 [115, 116]; Miller 1991 [250]; Mason and Muller 1982 [523]; McMahon 1994 [160]; Nascimento 2015 [524]; review, Keefe 2017 [525]). In a set of *in vitro* studies, Campenot reported that all neurite **growth** was controlled at the “nerve endings”:

> “[w]hen NGF was removed from the local environment of the distal portions of the neurites, the growth of these portions stopped (within three days) and [neurons] appeared to degenerate even though their somas and proximal portions were exposed to NGF” (Campenot 1977 [116])

Campenot found this result to be consistent with an earlier study published in 1975, also in sympathetic ganglia in juvenile rats, in which axotomization of sympathetic axons near the ganglion induced degeneration of those neurons, but “degeneration could be prevented by a period of intravenous (IV, *i.e.* systemic) NGF administration, which resulted in functional recovery of the innervation” (Hendry 1975 [122]).

Another nerve group, facial nerves, involves both sensory (sympathetic) and motor nerves in surgical incisions (Myckatyn and Mackinnon 2004 [526]). In 2017, investigators confirmed earlier findings, finding that axonal degeneration begins within 1 day (Huang 2017 [527]; Takaso 2020 [513]). In their report, Takaso *et al.* (Takaso 2020 [513]) noted that facial nerve axotomy may result in both retrograde (Akasawa 2004 [528]) and anterograde transport, or Wallerian degeneration, defined as “the process by which the distal nerve degenerates after axotomy and is cleared by phagocytosis” (Niemi 2013 [529]). Also in facial nerves, neurotrophins other than NGF, *i.e.,* ciliary neurotrophic factor (CNTF) may protect developing motor neurons from degeneration due to axotomy (Sendtner 1990 [530]).

Axotomization and regrowth have been reported in other studies in both mature and developing skin, in (i) mature skin, where the expression of TrkA receptors has been studied in adult rodents (Goodness 1997 [531]; Petruska and Mendell 2004 [532]); and (ii) developing skin, in trigeminal neurons (Huang 1999 [533]).

REFERENCES

1. Levi-Montalcini, R., *Effects of mouse tumor transplantation on the nervous system.* Ann., N. Y. Acad Sci, 1952(0077-8923 (Print)).
2. Hamburger, V. and R. Levi-Montalcini, *Proliferation, differentiation and degeneration in the spinal ganglia of the chick embryo under normal and experimental conditions.* J Exp Zool, 1949. **111**(3): p. 457-501.
3. Cohen, S., R. Levi-Montalcini, and V. Hamburger, *A NERVE GROWTH-STIMULATING FACTOR ISOLATED FROM SARCOMAS 37 AND 180.* Proc Natl Acad Sci U S A, 1954. **40**(10): p. 1014-8.
4. Levi-Montalcini, R., *The nerve growth factor: its role in growth, differentiation and function of the sympathetic adrenergic neuron.* Prog Brain Res, 1976. **45**: p. 235-58.
5. Aloe, L., *Rita Levi-Montalcini: the discovery of nerve growth factor and modern neurobiology.* Trends Cell Biol, 2004. **14**(7): p. 395-9.
6. Aloe, L., et al., *Nerve growth factor: from the early discoveries to the potential clinical use.* Journal of Translational Medicine, 2012. **10**(1): p. 239.
7. Rocco, M.L., et al., *Nerve Growth Factor: Early Studies and Recent Clinical Trials.* Curr Neuropharmacol, 2018. **16**(10): p. 1455-1465.
8. Neet, K.E. and R.B. Campenot, *Receptor binding, internalization, and retrograde transport of neurotrophic factors.* Cell Mol Life Sci, 2001. **58**(8): p. 1021-35.
9. Godfrey, E.W. and E.M. Shooter, *Nerve growth factor receptors on chick embryo sympathetic ganglion cells: binding characteristics and development.* J Neurosci, 1986. **6**(9): p. 2543-50.
10. Senger, D.L. and R.B. Campenot, *Rapid retrograde tyrosine phosphorylation of trkA and other proteins in rat sympathetic neurons in compartmented cultures.* J Cell Biol, 1997. **138**(2): p. 411-21.
11. Information, N.C.f.B. *NGF nerve growth factor [Canis lupus familiaris (dog)]* 2016 [cited 2016 30 August 2016]; Available from: http://www.ncbi.nlm.nih.gov/gene/403402.
12. KEGG. *Neurotrophin signaling pathway (type - pathway; taxonomic scope - conserved biosystem; BSID - 100064)*. KEGG source record [online database hosted on NCBI] 2021 [cited 2021 28 May 2021]; source record: ko04722]. Available from: https://www.ncbi.nlm.nih.gov/biosystems/100064?Sel=geneid:403402#show=genes.
13. Genomes, K.K.E.o.G.a. *Neurotrophin signaling pathway*. from KEGG source record: ko04722
Type: pathway
Taxonomic scope: conserved biosystem
BSID: 100064 2016 [cited 2016 30 August2016]; Available from: http://www.ncbi.nlm.nih.gov/biosystems/100064?Sel=geneid:403402#show=genes.
14. Luberg, K., et al., *Novel transcripts reveal a complex structure of the human TRKA gene and imply the presence of multiple protein isoforms.* BMC Neurosci, 2015. **16**: p. 78.
15. Fahnestock, M., et al., *The precursor pro-nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease.* Mol Cell Neurosci, 2001. **18**(2): p. 210-20.
16. Ioannou, M.S. and M. Fahnestock, *ProNGF, but Not NGF, Switches from Neurotrophic to Apoptotic Activity in Response to Reductions in TrkA Receptor Levels.* Int J Mol Sci, 2017. **18**(3).
17. Shekari, A. and M. Fahnestock, *Retrograde axonal transport of BDNF and proNGF diminishes with age in basal forebrain cholinergic neurons.* Neurobiol Aging, 2019. **84**: p. 131-140.
18. Kliemannel, M., et al., *The pro-peptide of proNGF: structure formation and intramolecular association with NGF.* Protein Sci, 2007. **16**(3): p. 411-9.
19. Peters, A., C.C. Proskauer, and I.R. Kaiserman-Abramof, *The small pyramidal neuron of the rat cerebral cortex. The axon hillock and initial segment.* J Cell Biol, 1968. **39**(3): p. 604-19.
20. Peters, A., *Connective tissue sheaths of peripheral nerves*. 3rd ed. The Fine Structure of the Nervous System: Neurons and Their Supporting Cells. 1991, New York, NY: Oxford University Press.
21. Echandia, E.L. and R.S. Piezzi, *Microtubules in the nerve fibers of the toad Bufo arenarum Hensel. Effect of low temperature on the sciatic nerve.* J Cell Biol, 1968. **39**(2): p. 491-7.
22. Burton, P.R., *Luminal material in microtubules of frog olfactory axons: structure and distribution.* J Cell Biol, 1984. **99**(2): p. 520-8.

23. Ure, D.R. and R.B. Campenot, *Retrograde transport and steady-state distribution of 125I-nerve growth factor in rat sympathetic neurons in compartmented cultures.* J Neurosci, 1997. **17**(4): p. 1282-90.
24. Garvalov, B.K., et al., *Luminal particles within cellular microtubules.* Journal of Cell Biology, 2006. **174**(6): p. 759-765.
25. Howe, C.L., et al., *NGF signaling from clathrin-coated vesicles: evidence that signaling endosomes serve as a platform for the Ras-MAPK pathway.* Neuron, 2001. **32**(5): p. 801-14.
26. Brown, A., *Axonal Transport*, in *Neuroscience in the 21st Century: From Basic to Clinical*, D.W. Pfaff, Editor. 2013, Springer New York: New York, NY. p. 255-308.
27. Brady, S.T., *BASIC PROPERTIES OF FAST AXONAL TRANSPORT AND THE ROLE OF FAST TRANSPORT IN AXONAL GROWTH*, in *Advances in Neurochemistry*. 1984.
28. Brady, S.T., *Axonal dynamics and regeneration*, in *Neuroregeneration*, A. Gorio, Editor. 1993, Raven Press: New York. p. 7-36.
29. Brady, S.T., *Axonal Transport Methods and Applications*, in *Neuromethods, General Neurochemical Techniques*, G.B.E. A. Boulton, Editor. 1985b, Humana Press: Clifton, NH. p. 419-476.
30. Treanor, J.J., et al., *Heterodimeric neurotrophins induce phosphorylation of Trk receptors and promote neuronal differentiation in PC12 cells.* J Biol Chem, 1995. **270**(39): p. 23104-10.
31. Butowt, R. and C.S. von Bartheld, *Fates of neurotrophins after retrograde axonal transport: phosphorylation of p75NTR is a sorting signal for delayed degradation.* J Neurosci, 2009. **29**(34): p. 10715-29.
32. Hendry, I.A., et al., *The retrograde axonal transport of nerve growth factor.* Brain Res, 1974a. **68**(1): p. 103-21.
33. Brimijoin, S. and L. Helland, *Rapid retrograde transport of dopamine-beta-hydroxylase as examined by the stop-flow technique.* Brain Res, 1976. **102**(2): p. 217-28.
34. Allen, R.D., et al., *Fast axonal transport in squid giant axon.* Science, 1982. **218**(4577): p. 1127-9.
35. Brady, S.T., R.J. Lasek, and R.D. Allen, *Fast axonal transport in extruded axoplasm from squid giant axon.* Science, 1982. **218**(4577): p. 1129-31.
36. Stenoien DL, B.S., *Fast Axonal Transport.*, in *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*, A.B. Siegel GJ, Albers RW, et al., editors, Editor. 1999, Lippincott-Raven: Philadelphia.
37. Avers, C.J., *Basic Cell Biology (2nd Ed.)*. 1982, Willard Grant Press: Boston. p. 229-236.
38. Sheeler, P. and D.E. Bianchi, *Cell Biology: Structure, Biochemistry and Function (2nd Ed)*, I. John Wiley & Sons, Editor. 1983: New York. p. 461-471.
39. Parton, R.G. and C.G. Dotti, *Cell biology of neuronal endocytosis.* 1993(0360-4012 (Print)).
40. Hosang, M. and E.M. Shooter, *Molecular characteristics of nerve growth factor receptors on PC12 cells.* J Biol Chem, 1985. **260**(1): p. 655-62.
41. Vissavajjhala, P., et al., *Structural domains of the extracellular domain of human nerve growth factor receptor detected by partial proteolysis.* Arch Biochem Biophys, 1992. **294**(1): p. 244-52.
42. Boutilier, J., et al., *Proneurotrophins require endocytosis and intracellular proteolysis to induce TrkA activation.* J Biol Chem, 2008. **283**(19): p. 12709-16.
43. Frampton, J.P., C. Guo, and B.A. Pierchala, *Expression of axonal protein degradation machinery in sympathetic neurons is regulated by nerve growth factor.* J Neurosci Res, 2012. **90**(8): p. 1533-46.
44. Vahrmeijer, A.L., et al., *Image-guided cancer surgery using near-infrared fluorescence.* Nature Reviews Clinical Oncology, 2013. **10**(9): p. 507-518.
45. Trouvilliez, S., C. Lagadec, and R.A. Toillon, *TrkA Co-Receptors: The Janus Face of TrkA?* Cancers (Basel), 2023. **15**(7).
46. Mandel, A.L., H. Ozdener, and V. Utermohlen, *Identification of pro- and mature brain-derived neurotrophic factor in human saliva.* Arch Oral Biol, 2009. **54**(7): p. 689-95.
47. Roy, S., *Seeing the unseen: the hidden world of slow axonal transport.* Neuroscientist, 2014. **20**(1): p. 71-81.
48. Heumann, R., *Regulation of the synthesis of nerve growth factor.* J Exp Biol, 1987a. **132**: p. 133-50.
49. Heumann, R., et al., *Changes of nerve growth factor synthesis in nonneuronal cells in response to sciatic nerve transection.* J Cell Biol, 1987b. **104**(6): p. 1623-31.
50. Acheson, A., et al., *Detection of brain-derived neurotrophic factor-like activity in fibroblasts and Schwann cells: inhibition by antibodies to NGF.* Neuron, 1991. **7**(2): p. 265-75.
51. Yoshida, K. and F.H. Gage, *Cooperative regulation of nerve growth factor synthesis and secretion in fibroblasts and astrocytes by fibroblast growth factor and other cytokines.* Brain Res, 1992. **569**(1): p. 14-25.

52. Ernfors, P., J.P. Merlio, and H. Persson, *Cells Expressing mRNA for Neurotrophins and their Receptors During Embryonic Rat Development.* Eur J Neurosci, 1992. **4**(11): p. 1140-1158.
53. Schecterson, L.C. and M. Bothwell, *Novel roles for neurotrophins are suggested by BDNF and NT-3 mRNA expression in developing neurons.* Neuron, 1992. **9**(3): p. 449-63.
54. Acheson, A., et al., *A BDNF autocrine loop in adult sensory neurons prevents cell death.* Nature, 1995. **374**(6521): p. 450-3.
55. Richardson, P.M., V.M. Issa, and R.J. Riopelle, *Distribution of neuronal receptors for nerve growth factor in the rat.* J Neurosci, 1986. **6**(8): p. 2312-21.
56. Yan, C., et al., *TrkA as a Life and Death Receptor.* Cancer Research, 2002. **62**(17): p. 4867.
57. Alastra, G., et al., *Nerve Growth Factor Biodelivery: A Limiting Step in Moving Toward Extensive Clinical Application?* Frontiers in Neuroscience, 2021. **15**: p. 879.
58. Micera, A., et al., *New insights on the involvement of Nerve Growth Factor in allergic inflammation and fibrosis.* Cytokine Growth Factor Rev, 2003. **14**(5): p. 369-74.
59. Lambiase, A., et al., *Nerve growth factor and the immune system: old and new concepts in the cross-talk between immune and resident cells during pathophysiological conditions.* Curr Opin Allergy Clin Immunol, 2004. **4**(5): p. 425-30.
60. Aloe, L., et al., *Nerve Growth Factor: A Focus on Neuroscience and Therapy.* Curr Neuropharmacol, 2015. **13**(3): p. 294-303.
61. Minnone, G., F. De Benedetti, and L. Bracci-Laudiero, *NGF and Its Receptors in the Regulation of Inflammatory Response.* Int J Mol Sci, 2017b. **18**(5).
62. Hempstead, B.L., *Deciphering proneurotrophin actions.* Handb Exp Pharmacol, 2014. **220**: p. 17-32.
63. Fahnestock, M. and A. Shekari, *ProNGF and Neurodegeneration in Alzheimer's Disease.* Front Neurosci, 2019. **13**: p. 129.
64. Fudalej, E., et al., *Neuroprotective Factors of the Retina and Their Role in Promoting Survival of Retinal Ganglion Cells: A Review.* Ophthalmic Res, 2021. **64**(3): p. 345-355.
65. Kahl, S.B., et al. *BDNF as targeting platform to deliver small molecules to the optic nerve: anti-inflammatory drugs to treat blast-induced traumatic optic neuropathy (b-iTON)*. in *Military Health System Research Symposium (MHSRS)*. 2019. Orlando, FL.
66. Toma, J.G., et al., *Spatial regulation of neuronal gene expression in response to nerve growth factor.* Dev Biol, 1997. **184**(1): p. 1-9.
67. Rodriguez-Tébar, A., G. Dechant, and Y.A. Barde, *Binding of brain-derived neurotrophic factor to the nerve growth factor receptor.* Neuron, 1990. **4**(4): p. 487-92.
68. Rodríguez-Tébar, A., et al., *Binding of neurotrophin-3 to its neuronal receptors and interactions with nerve growth factor and brain-derived neurotrophic factor.* EMBO J, 1992. **11**(3): p. 917-22.
69. Yancopoulos, G.D., et al., *Neurotrophic factors, their receptors, and the signal transduction pathways they activate.* Cold Spring Harb Symp Quant Biol, 1990. **55**: p. 371-9.
70. Soppet, D., et al., *The neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the trkB tyrosine kinase receptor.* Cell, 1991. **65**(5): p. 895-903.
71. Benedetti, I., et al., *Neuroanatomical and immunohistochemical studies on the dorsal neurons in the spinal cord of Trigla lucerna L. and Scorpaena porcus L. (Scorpaeniformes).* Ann Anat, 1993. **175**(1): p. 77-80.
72. Windisch, J.M., et al., *Brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4 bind to a single leucine-rich motif of TrkB.* Biochemistry, 1995. **34**(35): p. 11256-63.
73. Belliveau, D.J., et al., *NGF and neurotrophin-3 both activate TrkA on sympathetic neurons but differentially regulate survival and neuritogenesis.* J Cell Biol, 1997. **136**(2): p. 375-88.
74. Arévalo, J.C., et al., *A unique pathway for sustained neurotrophin signaling through an ankyrin-rich membrane-spanning protein.* EMBO J, 2004. **23**(12): p. 2358-68.
75. Jiang, T., et al., *Development of small-molecule tropomyosin receptor kinase (TRK) inhibitors for NTRK fusion cancers.* Acta Pharm Sin B, 2021. **11**(2): p. 355-372.
76. Johnson, E.M., Jr., R.Y. Andres, and R.A. Bradshaw, *Characterization of the retrograde transport of nerve growth factor (NGF) using high specific activity [125I] NGF.* Brain Res, 1978a. **150**(2): p. 319-31.
77. Johnson, E.M., Jr., et al., *Demonstration of the retrograde transport of nerve growth factor receptor in the peripheral and central nervous system.* J Neurosci, 1987. **7**(3): p. 923-9.
78. Martin-Zanca, D., et al., *Molecular and biochemical characterization of the human trk proto-oncogene.* Mol Cell Biol, 1989. **9**(1): p. 24-33.

79. Klein, R., et al., *trkB, a novel tyrosine protein kinase receptor expressed during mouse neural development.* EMBO J, 1989. **8**(12): p. 3701-9.
80. Klein, R., et al., *The trkB tyrosine protein kinase gene codes for a second neurogenic receptor that lacks the catalytic kinase domain.* Cell, 1990a. **61**(4): p. 647-56.
81. Klein, R., et al., *The trk proto-oncogene encodes a receptor for nerve growth factor.* Cell, 1991a. **65**(1): p. 189-97.
82. Klein, R., et al., *Expression of the tyrosine kinase receptor gene trkB is confined to the murine embryonic and adult nervous system.* Development, 1990b. **109**(4): p. 845-50.
83. Klein, R., et al., *The trkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3.* Cell, 1991b. **66**(2): p. 395-403.
84. Middlemas, D.S., R.A. Lindberg, and T. Hunter, *trkB, a neural receptor protein-tyrosine kinase: evidence for a full-length and two truncated receptors.* Mol Cell Biol, 1991. **11**(1): p. 143-53.
85. Squinto, S.P., et al., *trkB encodes a functional receptor for brain-derived neurotrophic factor and neurotrophin-3 but not nerve growth factor.* Cell, 1991. **65**(5): p. 885-93.
86. Martin-Zanca, D., S.H. Hughes, and M. Barbacid, *A human oncogene formed by the fusion of truncated tropomyosin and protein tyrosine kinase sequences.* Nature, 1986. **319**(6056): p. 743-8.
87. Lamballe, F., R. Klein, and M. Barbacid, *trkC, a new member of the trk family of tyrosine protein kinases, is a receptor for neurotrophin-3.* Cell, 1991a. **66**(5): p. 967-79.
88. Stöckel, K. and H. Thoenen, *Retrograde axonal transport of nerve growth factor: specificity and biological importance.* Brain Res, 1975. **85**(2): p. 337-41.
89. Goedert, M., et al., *Nerve growth factor mRNA in peripheral and central rat tissues and in the human central nervous system: lesion effects in the rat brain and levels in Alzheimer's disease.* Brain Res, 1986. **387**(1): p. 85-92.
90. Palmatier, M.A., B.K. Hartman, and E.M. Johnson, Jr., *Demonstration of retrogradely transported endogenous nerve growth factor in axons of sympathetic neurons.* J Neurosci, 1984. **4**(3): p. 751-6.
91. Korsching, S. and H. Thoenen, *Developmental changes of nerve growth factor levels in sympathetic ganglia and their target organs.* Dev Biol, 1988. **126**(1): p. 40-6.
92. Barde, Y.A., D. Edgar, and H. Thoenen, *Purification of a new neurotrophic factor from mammalian brain.* EMBO J, 1982. **1**(5): p. 549-53.
93. Cordon-Cardo, C., et al., *The trk tyrosine protein kinase mediates the mitogenic properties of nerve growth factor and neurotrophin-3.* Cell, 1991. **66**(1): p. 173-83.
94. Hempstead, B.L., et al., *High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor.* Nature, 1991. **350**(6320): p. 678-83.
95. Barbacid, M., et al., *The trk family of tyrosine protein kinase receptors.* Biochim Biophys Acta, 1991. **1072**(2-3): p. 115-27.
96. Roux, P.P. and P.A. Barker, *Neurotrophin signaling through the p75 neurotrophin receptor.* Prog Neurobiol, 2002. **67**(3): p. 203-33.
97. Wiesmann, C., et al., *Crystal structure of nerve growth factor in complex with the ligand-binding domain of the TrkA receptor.* Nature, 1999. **401**(6749): p. 184-8.
98. Ultsch, M.H., et al., *Crystal structures of the neurotrophin-binding domain of TrkA, TrkB and TrkC.* J Mol Biol, 1999. **290**(1): p. 149-59.
99. Loeb, D.M., et al., *The trk proto-oncogene rescues NGF responsiveness in mutant NGF-nonresponsive PC12 cell lines.* Cell, 1991. **66**(5): p. 961-6.
100. Loeb, D.M. and L.A. Greene, *Transfection with trk restores "slow" NGF binding, efficient NGF uptake, and multiple NGF responses to NGF-nonresponsive PC12 cell mutants.* J Neurosci, 1993. **13**(7): p. 2919-29.
101. Ibáñez, C.F., et al., *Disruption of the low affinity receptor-binding site in NGF allows neuronal survival and differentiation by binding to the trk gene product.* Cell, 1992. **69**(2): p. 329-41.
102. Meakin, S.O. and E.M. Shooter, *Molecular investigations on the high-affinity nerve growth factor receptor.* Neuron, 1991a. **6**(1): p. 153-63.
103. Meakin, S.O. and E.M. Shooter, *Tyrosine kinase activity coupled to the high-affinity nerve growth factor-receptor complex.* Proc Natl Acad Sci U S A, 1991b. **88**(13): p. 5862-6.
104. Meakin, S.O., et al., *The rat trk protooncogene product exhibits properties characteristic of the slow nerve growth factor receptor.* Proc Natl Acad Sci U S A, 1992. **89**(6): p. 2374-8.
105. Barker, P.A., et al., *The low affinity neurotrophin receptor, p75LNTR, is palmitoylated by thioester formation through cysteine 279.* J Biol Chem, 1994. **269**(48): p. 30645-50.

106. Barker, P.A., et al., *Tissue-specific alternative splicing generates two isoforms of the trkA receptor.* J Biol Chem, 1993. **268**(20): p. 15150-7.
107. Drinkwater, C.C., et al., *The carboxyl terminus of nerve growth factor is required for biological activity.* J Biol Chem, 1993. **268**(31): p. 23202-7.
108. Chao, M.V., et al., *Gene transfer and molecular cloning of the human NGF receptor.* Science, 1986. **232**(4749): p. 518-21.
109. Johnson, D., et al., *Expression and structure of the human NGF receptor.* Cell, 1986. **47**(4): p. 545-54.
110. Radeke, M.J., et al., *Gene transfer and molecular cloning of the rat nerve growth factor receptor.* Nature, 1987. **325**(6105): p. 593-7.
111. Burton, L.E., *The Production of Recombinant Neurotrophins*, in *Neuromethods, V. 25, Neurotrophic Factors*, G.B. A. Bolton, F. Hefti, Editor. 1993, The Humana Press. p. 155-175.
112. Espada, J. and J. Martín-Pérez, *An Update on Src Family of Nonreceptor Tyrosine Kinases Biology.* Int Rev Cell Mol Biol, 2017. **331**: p. 83-122.
113. Cohen, P., D. Cross, and P.A. Jänne, *Kinase drug discovery 20 years after imatinib: progress and future directions.* Nature Reviews Drug Discovery, 2021. **20**(7): p. 551-569.
114. Ip, N.Y., et al., *Similarities and differences in the way neurotrophins interact with the Trk receptors in neuronal and nonneuronal cells.* Neuron, 1993a. **10**(2): p. 137-49.
115. Campenot, R.B., *Local control of neurite development by nerve growth factor.* Proc Natl Acad Sci U S A, 1977. **74**(10): p. 4516-9.
116. Campenot, R.B., *Local control of neurite sprouting in cultured sympathetic neurons by nerve growth factor.* Brain Res, 1987. **465**(1-2): p. 293-301.
117. Campenot, R.B., *Development of sympathetic neurons in compartmentalized cultures. II. Local control of neurite survival by nerve growth factor.* Dev Biol, 1982a. **93**(1): p. 13-21.
118. Campenot, R.B., *NGF and the local control of nerve terminal growth.* J Neurobiol, 1994. **25**(6): p. 599-611.
119. Miller, F.D., et al., *Nerve growth factor derived from terminals selectively increases the ratio of p75 to trkA NGF receptors on mature sympathetic neurons.* Dev Biol, 1994. **161**(1): p. 206-17.
120. Levi-Montalcini, R. and L. Aloe, *Tropic, trophic, and transforming effects of nerve growth factor.* Adv Biochem Psychopharmacol, 1980. **25**: p. 3-15.
121. Stöckel, K., U. Paravicini, and H. Thoenen, *Specificity of the retrograde axonal transport of nerve growth factor.* Brain Res, 1974. **76**(3): p. 413-21.
122. Hendry, I.A., *The response of adrenergic neurones to axotomy and nerve growth factor.* Brain Res, 1975. **94**(1): p. 87-97.
123. Hendry, I.A., *Developmental changes in tissue and plasma concentrations of the biologically active species of nerve growth factor in the mouse, by using a two-site radioimmunoassay.* Biochem J, 1972. **128**(5): p. 1265-72.
124. Hendry, I.A., *Cell division in the developing sympathetic nervous system.* J Neurocytol, 1977. **6**(3): p. 299-309.
125. Hendry, I.A. and J. Campbell, *Morphometric analysis of rat superior cervical ganglion after axotomy and nerve growth factor treatment.* J Neurocytol, 1976. **5**(3): p. 351-60.
126. Hendry, I.A. and L.L. Iversen, *Reduction in the concentration of nerve growth factor in mice after sialectomy and castration.* Nature, 1973. **243**(5409): p. 550-4.
127. Iversen, L.L., K. Stöckel, and H. Thoenen, *Autoradiographic studies of the retrograde axonal transport of nerve growth factor in mouse sympathetic neurones.* Brain Research, 1975. **88**(1): p. 37-43.
128. Campenot, R.B., *Development of sympathetic neurons in compartmentalized cultures. I Local control of neurite growth by nerve growth factor.* Dev Biol, 1982a. **93**(1): p. 1-12.
129. Zareen, N. and L.A. Greene, *Protocol for culturing sympathetic neurons from rat superior cervical ganglia (SCG).* J Vis Exp, 2009(23).
130. Jackson, M. and W. Tourtellotte, *Neuron Culture from Mouse Superior Cervical Ganglion.* Bio Protoc, 2014. **4**(2).
131. Taylor, A.M., et al., *A microfluidic culture platform for CNS axonal injury, regeneration and transport.* Nat Methods, 2005. **2**(8): p. 599-605.
132. Taylor, A.M., S.W. Rhee, and N.L. Jeon, *Microfluidic chambers for cell migration and neuroscience research.* Methods Mol Biol, 2006. **321**: p. 167-77.
133. Park, J.W., et al., *Advances in microfluidics-based experimental methods for neuroscience research.* Lab Chip, 2013. **13**(4): p. 509-21.

134. Park, J.W., et al., *Microfluidic culture platform for neuroscience research.* Nat Protoc, 2006. **1**(4): p. 2128-36.
135. Barres, B.A., et al., *Immunological, morphological, and electrophysiological variation among retinal ganglion cells purified by panning.* Neuron, 1988. **1**(9): p. 791-803.
136. Barres, B.A. and B. Stevens, *Purifying and Culturing Neural Cells*. A Laboratory Manual, ed. B.A.B.a.B. Stevens. 2014, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. 205.
137. DiStefano, P.S., et al., *The neurotrophins BDNF, NT-3, and NGF display distinct patterns of retrograde axonal transport in peripheral and central neurons.* Neuron, 1992. **8**(5): p. 983-93.
138. Lindsay, R.M., et al., *Neurotrophic factors: from molecule to man.* Trends Neurosci, 1994. **17**(5): p. 182-90.
139. Anderson, K.D., et al., *Differential distribution of exogenous BDNF, NGF, and NT-3 in the brain corresponds to the relative abundance and distribution of high-affinity and low-affinity neurotrophin receptors.* J Comp Neurol, 1995. **357**(2): p. 296-317.
140. Mufson, E.J., et al., *Distribution and retrograde transport of trophic factors in the central nervous system: functional implications for the treatment of neurodegenerative diseases.* Prog Neurobiol, 1999. **57**(4): p. 451-84.
141. DiStefano, P.S. and R. Curtis, *Receptor mediated retrograde axonal transport of neurotrophic factors is increased after peripheral nerve injury.* Prog Brain Res, 1994. **103**: p. 35-42.
142. Curtis, R., et al., *Differential role of the low affinity neurotrophin receptor (p75) in retrograde axonal transport of the neurotrophins.* Neuron, 1995. **14**(6): p. 1201-11.
143. Kim, S.U., R. Hogue-Angeletti, and N.K. Gonatas, *Localization of nerve growth factor receptors in sympathetic neurons cultured in vitro.* Brain Res, 1979. **168**(3): p. 602-8.
144. Carbonetto, S. and R.W. Stach, *Localization of nerve growth factor bound to neurons growing nerve fibers in culture.* Brain Res, 1982. **255**(3): p. 463-73.
145. Rohrer, H. and Y.A. Barde, *Presence and disappearance of nerve growth factor receptors on sensory neurons in culture.* Dev Biol, 1982. **89**(2): p. 309-15.
146. Ross, A.H., et al., *Characterization of nerve growth factor receptor in neural crest tumors using monoclonal antibodies.* Proc Natl Acad Sci U S A, 1984. **81**(21): p. 6681-5.
147. Sonnenfeld, K.H., et al., *Nerve growth factor receptors on dissociated neurofibroma Schwann-like cells.* Cancer Res, 1986. **46**(3): p. 1446-52.
148. Zimmermann, A. and A. Sutter, *beta-Nerve growth factor (beta NGF) receptors on glial cells. Cell-cell interaction between neurones and Schwann cells in cultures of chick sensory ganglia.* Embo j, 1983. **2**(6): p. 879-85.
149. Chao, M.V., *The p75 neurotrophin receptor.* J Neurobiol, 1994. **25**(11): p. 1373-85.
150. Chao, M.V., *Retrograde transport redux.* Neuron, 2003a. **39**(1): p. 1-2.
151. Friedman, W.J. and L.A. Greene, *Neurotrophin signaling via Trks and p75.* Exp Cell Res, 1999. **253**(1): p. 131-42.
152. Roux, P.P., et al., *The p75 neurotrophin receptor activates Akt (protein kinase B) through a phosphatidylinositol 3-kinase-dependent pathway.* J Biol Chem, 2001. **276**(25): p. 23097-104.
153. Pryor S, et al., *NGF Causes TrkA to Specifically Attract Microtubules to Lipid Rafts.* PLoS ONE, 2012. **7 (4)**.
154. Lalli, G. and G. Schiavo, *Analysis of retrograde transport in motor neurons reveals common endocytic carriers for tetanus toxin and neurotrophin receptor p75NTR.* J Cell Biol, 2002. **156**(2): p. 233-9.
155. Bronfman, F.C., et al., *Ligand-induced internalization of the p75 neurotrophin receptor: a slow route to the signaling endosome.* J Neurosci, 2003. **23**(8): p. 3209-20.
156. Meeker, R.B. and K.S. Williams, *The p75 neurotrophin receptor: at the crossroad of neural repair and death.* Neural Regen Res, 2015. **10**(5): p. 721-5.
157. Huang, E.J. and L.F. Reichardt, *Neurotrophins: roles in neuronal development and function.* Annu Rev Neurosci, 2001. **24**: p. 677-736.
158. Korsching, S. and H. Thoenen, *Nerve growth factor in sympathetic ganglia and corresponding target organs of the rat: correlation with density of sympathetic innervation.* Proc Natl Acad Sci U S A, 1983b. **80**(11): p. 3513-6.
159. Mu, X., et al., *Neurotrophin receptor genes are expressed in distinct patterns in developing dorsal root ganglia.* J Neurosci, 1993. **13**(9): p. 4029-41.
160. McMahon, S.B., et al., *Expression and coexpression of Trk receptors in subpopulations of adult primary sensory neurons projecting to identified peripheral targets.* Neuron, 1994. **12**(5): p. 1161-71.

161. Muragaki, Y., et al., *Expression of trk receptors in the developing and adult human central and peripheral nervous system.* J Comp Neurol, 1995. **356**(3): p. 387-97.
162. Campenot, R.B., *NGF uptake and retrograde signaling mechanisms in sympathetic neurons in compartmented cultures.* Results Probl Cell Differ, 2009. **48**: p. 141-58.
163. Sutter, A., et al., *Nerve growth factor receptors. Characterization of two distinct classes of binding sites on chick embryo sensory ganglia cells.* J Biol Chem, 1979. **254**(13): p. 5972-82.
164. Hendry, I.A., R. Stach, and K. Herrup, *Characteristics of the retrograde axonal transport system for nerve growth factor in the sympathetic nervous system.* Brain Res, 1974b. **82**(1): p. 117-28.
165. Stöckel, K. and H. Thoenen, *Retrograde axonal transport of nerve growth factor: specificity and biological importance.* Brain Res, 1975a. **85**(2): p. 337-41.
166. Dumas, M., M.E. Schwab, and H. Thoenen, *Retrograde axonal transport of specific macromolecules as a tool for characterizing nerve terminal membranes.* J Neurobiol, 1979. **10**(2): p. 179-97.
167. Olender, E.J. and R.W. Stach, *Sequestration of 125I-labeled beta nerve growth factor by sympathetic neurons.* J Biol Chem, 1980. **255**(19): p. 9338-43.
168. Battleman, D.S., A.I. Geller, and M.V. Chao, *HSV-1 vector-mediated gene transfer of the human nerve growth factor receptor p75hNGFR defines high-affinity NGF binding.* J Neurosci, 1993. **13**(3): p. 941-51.
169. Loy, R., et al., *Regional CNS uptake of blood-borne nerve growth factor.* J Neurosci Res, 1994a. **39**(3): p. 339-46.
170. Loy, R., et al., *Retrograde axonal transport and lesion-induced upregulation of the TrkA high-affinity NGF receptor.* Exp Neurol, 1994b. **130**(2): p. 377-86.
171. Green, S.H. and L.A. Greene, *A single Mr approximately 103,000 125I-beta-nerve growth factor-affinity-labeled species represents both the low and high affinity forms of the nerve growth factor receptor.* J Biol Chem, 1986. **261**(32): p. 15316-26.
172. Hempstead, B.L., L.S. Schleifer, and M.V. Chao, *Expression of functional nerve growth factor receptors after gene transfer.* Science, 1989. **243**(4889): p. 373-5.
173. Pleasure, S.J., et al., *Introduction of nerve growth factor (NGF) receptors into a medulloblastoma cell line results in expression of high- and low-affinity NGF receptors but not NGF-mediated differentiation.* Proc Natl Acad Sci U S A, 1990. **87**(21): p. 8496-500.
174. Matsushima, H. and E. Bogenmann, *Terminal differentiation in neuroblastoma cells transfected with the NGF receptor gene when treated with NGF.* Prog Clin Biol Res, 1991. **366**: p. 227-33.
175. Bothwell, M., *Keeping track of neurotrophin receptors.* Cell, 1991. **65**(6): p. 915-8.
176. Chao, M.V., *Neurotrophin receptors: a window into neuronal differentiation.* Neuron, 1992. **9**(4): p. 583-93.
177. Lee, K.F., et al., *Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system.* Cell, 1992. **69**(5): p. 737-49.
178. von Bartheld, C.S., et al., *Retrograde transport of neurotrophins from the eye to the brain in chick embryos: roles of the p75NTR and trkB receptors.* J Neurosci, 1996. **16**(9): p. 2995-3008.
179. Ehlers, M.D., et al., *NGF-stimulated retrograde transport of trkA in the mammalian nervous system.* J Cell Biol, 1995. **130**(1): p. 149-56.
180. Yan, Q., et al., *Influences of neurotrophins on mammalian motoneurons in vivo.* J Neurobiol, 1993. **24**(12): p. 1555-77.
181. Curtis, R. and P.S. DiStefano, *Neurotropic factors, retrograde axonal transport and cell signalling.* Trends Cell Biol, 1994. **4**(11): p. 383-6.
182. Xu, Y., et al., *Endocytosis and membrane receptor internalization: implication of F-BAR protein Carom.* Front Biosci (Landmark Ed), 2017. **22**: p. 1439-1457.
183. Cooper, G.M., *Microtubules*, in *The Cell: A Molecular Approach*, G.M. Cooper, Editor. 2000, Sinauer Associates: Sunderland, MA.
184. Morell P., Q.R.H., *Characteristic Composition of Myelin*, in *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*, A.B. Siegel GJ, Albers RW, et al., editors, Editor. 1999, Lippincott-Raven: Philadelphia.
185. Campenot, R.B., *NGF uptake and retrograde signaling mechanisms in sympathetic neurons in compartmented cultures.* Results Probl Cell Differ, 2009b. **48**: p. 141-58.
186. Straubinger, R.M., et al., *Endocytosis of liposomes and intracellular fate of encapsulated molecules: Encounter with a low pH compartment after internalization in coated vesicles.* Cell, 1983. **32**(4): p. 1069-1079.

187. Murthy, V.N. and C.F. Stevens, *Synaptic vesicles retain their identity through the endocytic cycle.* Nature, 1998. **392**(6675): p. 497-501.
188. McMahon, H.T. and E. Boucrot, *Molecular mechanism and physiological functions of clathrin-mediated endocytosis.* Nat Rev Mol Cell Biol, 2011. **12**(8): p. 517-33.
189. Du Toit, A., *Endocytosis. A new gateway into cells.* Nat Rev Mol Cell Biol, 2015. **16**(2): p. 68.
190. Roney, J.C., X.T. Cheng, and Z.H. Sheng, *Neuronal endolysosomal transport and lysosomal functionality in maintaining axonostasis.* J Cell Biol, 2022. **221**(3).
191. Brown, A., *Axonal transport of membranous and nonmembranous cargoes: a unified perspective.* J Cell Biol, 2003. **160**(6): p. 817-21.
192. Condon, K.H. and M.D. Ehlers, *CHAPTER 8 - Postsynaptic Machinery for Receptor Trafficking*, in *Protein Trafficking in Neurons*, A.J. Bean, Editor. 2007, Academic Press: Burlington. p. 143-174.
193. Gindhart, J.G. and K.P. Weber, *Lysosome and Endosome Organization and Transport in Neurons*, in *Encyclopedia of Neuroscience*, L.R. Squire, Editor. 2009, Academic Press: Oxford. p. 581-587.
194. Ye, H., et al., *Evidence in support of signaling endosome-based retrograde survival of sympathetic neurons.* Neuron, 2003. **39**(1): p. 57-68.
195. Marlin, M.C. and G. Li, *Biogenesis and function of the NGF/TrkA signaling endosome.* Int Rev Cell Mol Biol, 2015. **314**: p. 239-57.
196. Lehigh, K.M., K.M. West, and D.D. Ginty, *Retrogradely Transported TrkA Endosomes Signal Locally within Dendrites to Maintain Sympathetic Neuron Synapses.* Cell Rep, 2017. **19**(1): p. 86-100.
197. Barford, K., C. Deppmann, and B. Winckler, *The neurotrophin receptor signaling endosome: Where trafficking meets signaling.* Dev Neurobiol, 2017. **77**(4): p. 405-418.
198. Hu, Y.B., et al., *The endosomal-lysosomal system: from acidification and cargo sorting to neurodegeneration.* Transl Neurodegener, 2015. **4**: p. 18.
199. Ye, M., K.M. Lehigh, and D.D. Ginty, *Multivesicular bodies mediate long-range retrograde NGF-TrkA signaling.* Elife, 2018. **7**.
200. nanoComposix, F. 2023.
201. Muster, C.J., et al., *Physical mapping of adeno-associated virus serotype 4 DNA.* J Virol, 1980. **35**(3): p. 653-61.
202. Erickson, H.P., *Size and shape of protein molecules at the nanometer level determined by sedimentation, gel filtration, and electron microscopy.* Biol Proced Online, 2009. **11**: p. 32-51.
203. Paul, D., et al., *Phagocytosis dynamics depends on target shape.* Biophys J, 2013. **105**(5): p. 1143-50.
204. Baranov, M.V., et al., *Modulation of Immune Responses by Particle Size and Shape.* Front Immunol, 2020. **11**: p. 607945.
205. Kaksonen, M. and A. Roux, *Mechanisms of clathrin-mediated endocytosis.* Nature Reviews Molecular Cell Biology, 2018. **19**(5): p. 313-326.
206. Frey, W.H., et al., *Delivery of 125I-NGF to the Brain via the Olfactory Route.* Drug Delivery, 1997. **4**(2): p. 87-92.
207. Rosenfeld, R.D., et al., *Purification and identification of brain-derived neurotrophic factor from human serum.* Protein Expr Purif, 1995. **6**(4): p. 465-71.
208. Silhol, M., et al., *Age-related changes in brain-derived neurotrophic factor and tyrosine kinase receptor isoforms in the hippocampus and hypothalamus in male rats.* Neuroscience, 2005. **132**(3): p. 613-24.
209. Otani, K., M. Okada, and H. Yamawaki, *Diverse distribution of tyrosine receptor kinase B isoforms in rat multiple tissues.* J Vet Med Sci, 2017. **79**(9): p. 1516-1523.
210. Kordower, J.H., et al., *Intravenous administration of a transferrin receptor antibody-nerve growth factor conjugate prevents the degeneration of cholinergic striatal neurons in a model of Huntington disease.* Proc Natl Acad Sci U S A, 1994. **91**(19): p. 9077-80.
211. Charles, V., et al., *Atrophy of cholinergic basal forebrain neurons following excitotoxic cortical lesions is reversed by intravenous administration of an NGF conjugate.* Brain Res, 1996. **728**(2): p. 193-203.
212. Yue, J., et al., *Fluorescence-labeled immunomicelles: preparation, in vivo biodistribution, and ability to cross the blood-brain barrier.* Macromol Biosci, 2012. **12**(9): p. 1209-19.
213. Pillay, S., et al., *Adeno-associated Virus (AAV) Serotypes Have Distinctive Interactions with Domains of the Cellular AAV Receptor.* J Virol, 2017. **91**(18).
214. Tuszynski, M.H., et al., *Gene therapy in the adult primate brain: intraparenchymal grafts of cells genetically modified to produce nerve growth factor prevent cholinergic neuronal degeneration.* Gene Ther, 1996. **3**(4): p. 305-14.

215. Granholm, A.C., et al., *A non-invasive system for delivering neural growth factors across the blood-brain barrier: a review.* Rev Neurosci, 1998. **9**(1): p. 31-55.
216. Tuszynski, M.H., et al., *S3–04–02: NGF gene therapy.* Alzheimer's & Dementia, 2006. **2**(3S_Part_2): p. S50-S50.
217. Tuszynski, M.H., *Nerve growth factor gene therapy in Alzheimer disease.* Alzheimer Dis Assoc Disord, 2007. **21**(2): p. 179-89.
218. Nagahara, A.H. and M.H. Tuszynski, *Potential therapeutic uses of BDNF in neurological and psychiatric disorders.* Nat Rev Drug Discov, 2011. **10**(3): p. 209-19.
219. Bartus, R.T., *Translating the therapeutic potential of neurotrophic factors to clinical 'proof of concept': a personal saga achieving a career-long quest.* Neurobiol Dis, 2012. **48**(2): p. 153-78.
220. Bartus, R.T., et al., *Advancing neurotrophic factors as treatments for age-related neurodegenerative diseases: developing and demonstrating "clinical proof-of-concept" for AAV-neurturin (CERE-120) in Parkinson's disease.* Neurobiol Aging, 2013. **34**(1): p. 35-61.
221. Rafii, M.S., et al., *A phase1 study of stereotactic gene delivery of AAV2-NGF for Alzheimer's disease.* Alzheimers Dement, 2014. **10**(5): p. 571-81.
222. Rafii, M.S., et al., *Adeno-Associated Viral Vector (Serotype 2)-Nerve Growth Factor for Patients With Alzheimer Disease: A Randomized Clinical Trial.* JAMA Neurol, 2018. **75**(7): p. 834-841.
223. Govindasamy, L., et al., *Structural insights into adeno-associated virus serotype 5.* J Virol, 2013. **87**(20): p. 11187-99.
224. Tang, X., et al., *Anti-transferrin receptor-modified amphotericin B-loaded PLA-PEG nanoparticles cure Candidal meningitis and reduce drug toxicity.* Int J Nanomedicine, 2015. **10**: p. 6227-41.
225. Amani, H., et al., *Selenium nanoparticles for targeted stroke therapy through modulation of inflammatory and metabolic signaling.* Sci Rep, 2019. **9**(1): p. 6044.
226. Nguyen, C.B., et al., *Tissue disposition and pharmacokinetics of recombinant human nerve growth factor after acute and chronic subcutaneous administration in monkeys.* Drug Metab Dispos, 2000a. **28**(5): p. 598-607.
227. Nguyen, C.B., et al., *Stability and interactions of recombinant human nerve growth factor in different biological matrices: in vitro and in vivo studies.* Drug Metab Dispos, 2000b. **28**(5): p. 590-7.
228. Kaplan, D.R., D. Martin-Zanca, and L.F. Parada, *Tyrosine phosphorylation and tyrosine kinase activity of the trk proto-oncogene product induced by NGF.* Nature, 1991b. **350**(6314): p. 158-60.
229. Kim, U.H., et al., *Nerve growth factor stimulates phosphorylation of phospholipase C-gamma in PC12 cells.* J Biol Chem, 1991. **266**(3): p. 1359-62.
230. Vetter, M.L., et al., *Nerve growth factor rapidly stimulates tyrosine phosphorylation of phospholipase C-gamma 1 by a kinase activity associated with the product of the trk protooncogene.* Proc Natl Acad Sci U S A, 1991. **88**(13): p. 5650-4.
231. Kaplan, D.R., et al., *The trk proto-oncogene product: a signal transducing receptor for nerve growth factor.* Science, 1991a. **252**(5005): p. 554-8.
232. Jing, S., P. Tapley, and M. Barbacid, *Nerve growth factor mediates signal transduction through trk homodimer receptors.* Neuron, 1992. **9**(6): p. 1067-79.
233. Watson, F.L., et al., *Rapid nuclear responses to target-derived neurotrophins require retrograde transport of ligand-receptor complex.* J Neurosci, 1999. **19**(18): p. 7889-900.
234. Miller, F.D. and D.R. Kaplan, *On Trk for retrograde signaling.* 2001(0896-6273 (Print)).
235. Sofroniew, M.V., C.L. Howe, and W.C. Mobley, *Nerve growth factor signaling, neuroprotection, and neural repair.* Annu Rev Neurosci, 2001. **24**: p. 1217-81.
236. Hendry, I.A., Crouch, M., *Synergy, retrograde transport, and cell death*, in *Neurotrophic Factors*, e. S.E. Loughlin and J.H. Fallon, Editor. 1993, Academic: Boston. p. 51-88.
237. Purves, D., W.D. Snider, and J.T. Voyvodic, *Trophic regulation of nerve cell morphology and innervation in the autonomic nervous system.* Nature, 1988. **336**(6195): p. 123-128.
238. Barde, Y.A., *Trophic factors and neuronal survival.* Neuron, 1989. **2**(6): p. 1525-34.
239. Zweifel, L.S., R. Kuruvilla, and D.D. Ginty, *Functions and mechanisms of retrograde neurotrophin signalling.* Nat Rev Neurosci, 2005. **6**(8): p. 615-25.
240. Oppenheim RW, v.B.C., *Programmed cell death and neurotrophic factors* in *Fundamental neuroscience*, B.D. Squire L.R., Bloom F., du Lac S., Ghosh A., Editor. 2008, Elsevier: San Diego. p. 437-467.
241. MacInnis, B.L. and R.B. Campenot, *Retrograde support of neuronal survival without retrograde transport of nerve growth factor.* Science, 2002. **295**(5559): p. 1536-9.

242. Chao, M.V., *Neurotrophins and their receptors: a convergence point for many signalling pathways.* Nat Rev Neurosci, 2003b. **4**(4): p. 299-309.
243. Mok, S.A. and R.B. Campenot, *A nerve growth factor-induced retrograde survival signal mediated by mechanisms downstream of TrkA.* Neuropharmacology, 2007. **52**(2): p. 270-8.
244. Caterina, M.J., et al., *The capsaicin receptor: a heat-activated ion channel in the pain pathway.* Nature, 1997. **389**(6653): p. 816-24.
245. Zhu, W. and G.S. Oxford, *Phosphoinositide-3-kinase and mitogen activated protein kinase signaling pathways mediate acute NGF sensitization of TRPV1.* Mol Cell Neurosci, 2007. **34**(4): p. 689-700.
246. Conroy, J.N. and E.J. Coulson, *High-affinity TrkA and p75 neurotrophin receptor complexes: A twisted affair.* J Biol Chem, 2022. **298**(3): p. 101568.
247. Levi-Montalcini, R., *The nerve growth factor 35 years later.* Science, 1987. **237**(4819): p. 1154-62.
248. Levi-Montalcini, R., et al., *Nerve growth factor: from neurotrophin to neurokine.* Trends Neurosci, 1996. **19**(11): p. 514-20.
249. Mathew, T.C. and F.D. Miller, *Increased expression of T alpha 1 alpha-tubulin mRNA during collateral and NGF-induced sprouting of sympathetic neurons.* Dev Biol, 1990. **141**(1): p. 84-92.
250. Miller, F.D., T.C. Mathew, and J.G. Toma, *Regulation of nerve growth factor receptor gene expression by nerve growth factor in the developing peripheral nervous system.* J Cell Biol, 1991. **112**(2): p. 303-12.
251. Ma, Y., R.B. Campenot, and F.D. Miller, *Concentration-dependent regulation of neuronal gene expression by nerve growth factor.* J Cell Biol, 1992. **117**(1): p. 135-41.
252. Wyatt, S. and A.M. Davies, *Regulation of nerve growth factor receptor gene expression in sympathetic neurons during development.* J Cell Biol, 1995. **130**(6): p. 1435-46.
253. Bibel, M. and Y.A. Barde, *Neurotrophins: key regulators of cell fate and cell shape in the vertebrate nervous system.* Genes Dev, 2000. **14**(23): p. 2919-37.
254. Poo, M.M., *Neurotrophins as synaptic modulators.* Nat Rev Neurosci, 2001. **2**(1): p. 24-32.
255. Huang, E.J. and L.F. Reichardt, *Trk receptors: roles in neuronal signal transduction.* Annu Rev Biochem, 2003. **72**: p. 609-42.
256. Burton, P.R. and J.L. Paige, *Polarity of axoplasmic microtubules in the olfactory nerve of the frog.* Proc Natl Acad Sci U S A, 1981. **78**(5): p. 3269-73.
257. Heidemann, S.R., J.M. Landers, and M.A. Hamborg, *Polarity orientation of axonal microtubules.* J Cell Biol, 1981. **91**(3 Pt 1): p. 661-5.
258. Heidemann, S.R., et al., *Spatial organization of axonal microtubules.* J Cell Biol, 1984. **99**(4 Pt 1): p. 1289-95.
259. Yano, H. and M.V. Chao, *Neurotrophin receptor structure and interactions.* Pharm Acta Helv, 2000. **74**(2-3): p. 253-60.
260. Delcroix, J.-D., et al., *NGF Signaling in Sensory Neurons: Evidence that Early Endosomes Carry NGF Retrograde Signals.* Neuron, 2003. **39**(1): p. 69-84.
261. Miaczynska, M., L. Pelkmans, and M. Zerial, *Not just a sink: endosomes in control of signal transduction.* Curr Opin Cell Biol, 2004. **16**(4): p. 400-6.
262. Heerssen, H.M. and R.A. Segal, *Location, location, location: a spatial view of neurotrophin signal transduction.* Trends Neurosci, 2002. **25**(3): p. 160-5.
263. Wu, C., et al., *A functional dynein-microtubule network is required for NGF signaling through the Rap1/MAPK pathway.* Traffic, 2007. **8**(11): p. 1503-20.
264. Mitchell, D.J., et al., *Trk activation of the ERK1/2 kinase pathway stimulates intermediate chain phosphorylation and recruits cytoplasmic dynein to signaling endosomes for retrograde axonal transport.* J Neurosci, 2012. **32**(44): p. 15495-510.
265. Courchet, J., et al., *Terminal axon branching is regulated by the LKB1-NUAK1 kinase pathway via presynaptic mitochondrial capture.* Cell, 2013. **153**(7): p. 1510-25.
266. Greif, K.F., et al., *Synaptotagmin-1 promotes the formation of axonal filopodia and branches along the developing axons of forebrain neurons.* Dev Neurobiol, 2013. **73**(1): p. 27-44.
267. Zala, D., M.V. Hinckelmann, and F. Saudou, *Huntingtin's function in axonal transport is conserved in Drosophila melanogaster.* PLoS One, 2013. **8**(3): p. e60162.
268. Hinckelmann, M.V., D. Zala, and F. Saudou, *Releasing the brake: restoring fast axonal transport in neurodegenerative disorders.* Trends Cell Biol, 2013. **23**(12): p. 634-43.
269. Hinckelmann, M.V., et al., *Self-propelling vesicles define glycolysis as the minimal energy machinery for neuronal transport.* Nat Commun, 2016. **7**: p. 13233.

270. Sainath, R. and G. Gallo, *Bioenergetic Requirements and Spatiotemporal Profile of Nerve Growth Factor Induced PI3K-Akt Signaling Along Sensory Axons.* Front Mol Neurosci, 2021. **14**: p. 726331.
271. Dunn, W.A. and A.L. Hubbard, *Receptor-mediated endocytosis of epidermal growth factor by hepatocytes in the perfused rat liver: ligand and receptor dynamics.* J Cell Biol, 1984. **98**(6): p. 2148-59.
272. Schmid, S.L., et al., *Two distinct subpopulations of endosomes involved in membrane recycling and transport to lysosomes.* Cell, 1988. **52**(1): p. 73-83.
273. Stoorvogel, W., et al., *Late endosomes derive from early endosomes by maturation.* Cell, 1991. **65**(3): p. 417-27.
274. Grafstein, B. and D.S. Forman, *Intracellular transport in neurons.* Physiol Rev, 1980. **60**(4): p. 1167-283.
275. Weiss, P. and H.B. Hiscoe, *Experiments on the mechanism of nerve growth.* J Exp Zool, 1948. **107**(3): p. 315-95.
276. LaVail, J.H. and M.M. LaVail, *Retrograde axonal transport in the central nervous system.* Science, 1972. **176**(4042): p. 1416-7.
277. Honig, M.G. and R.I. Hume, *Dil and diO: versatile fluorescent dyes for neuronal labelling and pathway tracing.* Trends Neurosci, 1989. **12**(9): p. 333-5, 340-1.
278. Kristensson, K., Y. Olsson, and J. Sjöstrand, *Axonal uptake and retrograde transport of exogenous proteins in the hypoglossal nerve.* Brain Res, 1971. **32**(2): p. 399-406.
279. Schwab, M.E. and H. Thoenen, *Retrograde axonal and transsynaptic transport of macromolecules: physiological and pathophysiological importance.* Agents Actions, 1977. **7**(3): p. 361-8.
280. Schwab, M.E., et al., *Nerve growth factor (NGF) in the rat CNS: absence of specific retrograde axonal transport and tyrosine hydroxylase induction in locus coeruleus and substantia nigra.* Brain Res, 1979. **168**(3): p. 473-83.
281. Schwab, M.E., *[Axonal transport from the nerve ending to the nerve cell body: a pathway for trophic signals and neurotoxins].* Bull Schweiz Akad Med Wiss, 1980. **36**(1-3): p. 7-19.
282. Thoenen, H. and Y.A. Barde, *Physiology of nerve growth factor.* Physiol Rev, 1980. **60**(4): p. 1284-335.
283. Claude, P., et al., *Binding, internalization, and retrograde transport of 125I-nerve growth factor in cultured rat sympathetic neurons.* J Neurosci, 1982b. **2**(4): p. 431-42.
284. Schwab, M.E., R. Heumann, and H. Thoenen, *Communication between target organs and nerve cells: retrograde axonal transport and site of action of nerve growth factors.* Cold Spring Harb Symp Quant Biol, 1982. **46 Pt 1**: p. 125-34.
285. Bisby, M.A., *Functions of retrograde axonal transport.* Fed Proc, 1982. **41**(7): p. 2307-11.
286. Schwab ME, T.H., *Retrograde axonal transport*, in *Handbook of neurochemistry*. 1983, Plenum: New York. p. 381-404.
287. Korsching, S. and H. Thoenen, *Quantitative demonstration of the retrograde axonal transport of endogenous nerve growth factor.* Neurosci Lett, 1983a. **39**(1): p. 1-4.
288. Ross, A.H., et al., *Axonal transport of the trkA high-affinity NGF receptor.* Prog Brain Res, 1994. **103**: p. 15-21.
289. Vallee, R.B. and G.S. Bloom, *Mechanisms of fast and slow axonal transport.* Annu Rev Neurosci, 1991. **14**: p. 59-92.
290. Campenot, R.B. and H. Eng, *Protein synthesis in axons and its possible functions.* J Neurocytol, 2000. **29**(11-12): p. 793-8.
291. Campenot, R.B., et al., *Block of slow axonal transport and axonal growth by brefeldin A in compartmented cultures of rat sympathetic neurons.* Neuropharmacology, 2003. **44**(8): p. 1107-17.
292. Gibbs, K.L., et al., *In vivo imaging of axonal transport in murine motor and sensory neurons.* J Neurosci Methods, 2015. **257**: p. 26-33.
293. Sleigh J, S.G. *Older but not slower: aging does not alter axonal transport dynamics of signalling endosomes in vivo*. Matters. 2 (6) 2016 [cited 2021 6 August 2021].
294. Stöckel, K., M. Schwab, and H. Thoenen, *Specificity of retrograde transport of nerve growth factor (NGF) in sensory neurons: a biochemical and morphological study.* Brain Res, 1975b. **89**(1): p. 1-14.
295. Yip, H.K. and E.M. Johnson, Jr., *Comparative dynamics of retrograde transport of nerve growth factor and horseradish peroxidase in rat lumbar dorsal root ganglia.* J Neurocytol, 1986. **15**(6): p. 789-98.
296. Brady, S.T., R.J. Lasek, and R.D. Allen, *Fast axonal transport in extruded axoplasm from squid giant axon.* Science (New York, N.Y.), 1982. **218**(4577): p. 1129-1131.
297. Brady, S.T., *A novel brain ATPase with properties expected for the fast axonal transport motor.* Nature, 1985a. **317**(6032): p. 73-5.

298. Brady, S.T., *Molecular motors in the nervous system.* Neuron, 1991. **7**(4): p. 521-33.
299. Bloom, G.S., F.C. Luca, and R.B. Vallee, *Widespread cellular distribution of MAP-1A (microtubule-associated protein 1A) in the mitotic spindle and on interphase microtubules.* J Cell Biol, 1984. **98**(1): p. 331-40.
300. Paschal, B.M. and R.B. Vallee, *Retrograde transport by the microtubule-associated protein MAP 1C.* Nature, 1987a. **330**(6144): p. 181-3.
301. Paschal, B.M., H.S. Shpetner, and R.B. Vallee, *MAP 1C is a microtubule-activated ATPase which translocates microtubules in vitro and has dynein-like properties.* J Cell Biol, 1987b. **105**(3): p. 1273-82.
302. Leopold, P.L., et al., *Nucleotide specificity for the bidirectional transport of membrane-bounded organelles in isolated axoplasm.* Cell Motil Cytoskeleton, 1990. **15**(4): p. 210-9.
303. Stenoien DL, B.S., *Discovery and Conceptual Development of Fast and Slow Axonal Transport*, in *Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 6th edition.* , A.B. Siegel GJ, Albers RW, et al., editors, Editor. 1999, Lippincott-Raven: Philadelphia.
304. Aboud, O., et al., *Aging, Alzheimer's, and APOE genotype influence the expression and neuronal distribution patterns of microtubule motor protein dynactin-P50.* Front Cell Neurosci, 2015. **9**: p. 103.
305. Yankner, B.A. and E.M. Shooter, *Nerve growth factor in the nucleus: interaction with receptors on the nuclear membrane.* Proc Natl Acad Sci U S A, 1979. **76**(3): p. 1269-73.
306. Landreth, G.E. and E.M. Shooter, *Nerve growth factor receptors on PC12 cells: ligand-induced conversion from low- to high-affinity states.* Proc Natl Acad Sci U S A, 1980. **77**(8): p. 4751-5.
307. Schechter, A.L. and M.A. Bothwell, *Nerve growth factor receptors on PC12 cells: evidence for two receptor classes with differing cytoskeletal association.* Cell, 1981. **24**(3): p. 867-74.
308. Lasek, R.J., Brady, S.T., *The structural hypothesis of axonal transport: Two classes of moving elements*, in *Axoplasmic Transport*, e. D.G. Weiss, Editor. 1982, Springer-Verlag: Berlin, Heidelberg, New York. p. 397-405.
309. Tytell, M., et al., *Axonal transport: each major rate component reflects the movement of distinct macromolecular complexes.* Science, 1981. **214**(4517): p. 179-81.
310. Baitinger, C., Levine, J., Lorenz, T., Simon, C., Skene, P., and Willard, M., *Characteristics of axonally transported proteins*, in *Axoplasmic Transport*, e. D.G. Weiss, Editor. 1982, Springer-Verlag: Berlin, Heidelberg, New York. p. 110-120.
311. Baitinger, C., et al., *Axonally transported proteins in axon development, maintenance, and regeneration.* Cold Spring Harb Symp Quant Biol, 1983. **48 Pt 2**: p. 791-802.
312. Elluru, R.G., G.S. Bloom, and S.T. Brady, *Fast axonal transport of kinesin in the rat visual system: functionality of kinesin heavy chain isoforms.* Mol Biol Cell, 1995. **6**(1): p. 21-40.
313. Pirahanchi, Y., Jessu, R., Aeddula, N.R., *Physiology, Sodium Potassium Pump*. 2023, StatPearls Publishing: Treasure Island, FL.
314. Xu, T., V. Pagadala, and D.M. Mueller, *Understanding structure, function, and mutations in the mitochondrial ATP synthase.* Microb Cell, 2015. **2**(4): p. 105-125.
315. Twelvetrees, A.E., et al., *The Dynamic Localization of Cytoplasmic Dynein in Neurons Is Driven by Kinesin-1.* Neuron, 2016. **90**(5): p. 1000-15.
316. Guillaud, L., et al., *Anterograde Axonal Transport in Neuronal Homeostasis and Disease.* Front Mol Neurosci, 2020. **13**: p. 556175.
317. Dillman, J.F., 3rd, et al., *Functional analysis of dynactin and cytoplasmic dynein in slow axonal transport.* J Neurosci, 1996a. **16**(21): p. 6742-52.
318. Dillman, J.F., 3rd, L.P. Dabney, and K.K. Pfister, *Cytoplasmic dynein is associated with slow axonal transport.* Proc Natl Acad Sci U S A, 1996b. **93**(1): p. 141-4.
319. Lasek, R.J., *Polymer sliding in axons.* J Cell Sci Suppl, 1986. **5**: p. 161-79.
320. Vale, R.D., Banker, G. & Hall, Z. W. (1992) in An Introduction to Molecular Neurobiology, ed. Hall, Z. W. (Sinauer, Sunderland, MA), pp. 247-280., *The neuronal cytoskeleton*, in *An Introduction to Molecular Neurobiology*, Z.W. Hall, Editor. 1992, Sinauer Associates, Inc.: Sunderland, MA. p. 247-280.
321. Smith, R.S. and P.D. Cooper, *Variability and temperature dependence of the velocity of retrograde particle transport in myelinated axons.* Can J Physiol Pharmacol, 1981. **59**(8): p. 857-63.
322. Tsukita, S. and H. Ishikawa, *The movement of membranous organelles in axons. Electron microscopic identification of anterogradely and retrogradely transported organelles.* J Cell Biol, 1980. **84**(3): p. 513-30.
323. Brady, S.T., *Neuromethods (book)*, in *Neuromethods*, A.A.B. Boulton, G.B., Editor. 1985c, Humana: Clifton, NJ. p. 419-473.

324. Paschal, B.M., H.S. Shpetner, and R.B. Vallee, *MAP 1C is a microtubule-activated ATPase which translocates microtubules in vitro and has dynein-like properties.* J Cell Biol, 1987. **105**(3): p. 1273-82.
325. Schnapp, B.J. and T.S. Reese, *Dynein is the motor for retrograde axonal transport of organelles.* Proc Natl Acad Sci U S A, 1989. **86**(5): p. 1548-52.
326. Bhabha, G., et al., *How Dynein Moves Along Microtubules.* Trends Biochem Sci, 2016. **41**(1): p. 94-105.
327. Can, S., et al., *Directionality of dynein is controlled by the angle and length of its stalk.* Nature, 2019. **566**(7744): p. 407-410.
328. Canty, J.T., et al., *Structure and Mechanics of Dynein Motors.* Annu Rev Biophys, 2021. **50**: p. 549-574.
329. Vallee, R.B. and G.S. Bloom, *Mechanisms of fast and slow axonal transport.* 1991(0147-006X (Print)).
330. Bunke, D., U. Monning, R.M. Krypta, S.A. Courteneidge, C.L. Masters, and K.T. Beyreuther, *Tyrosine phosphorylation of the cytoplasmic domain of Alzheimer protein precursors*, in *Alzheimer Disease: Basic Mechanisms, Diagnosis and Therapeutic Strategies*, D.R.C.M. K. Iqbal, B. Winblad, and H.M. Wisniewski, Editor. 1991, Wiley: New York. p. 229-235.
331. Iqbal, K.a.G.-I., I., *Alzheimer Disease: from cytoskeletal protein pathology to neuronal degeneration*, in *Alzheimer Disease: Basic Mechanisms, Diagnosis and Therapeutic Strategies*, D.R.C.M. K. Iqbal, B. Winblad, and H.M. Wisniewski, Editor. 1991, Wiley: New York. p. 173-180.
332. Tang, Y., et al., *Fast vesicle transport is required for the slow axonal transport of synapsin.* J Neurosci, 2013. **33**(39): p. 15362-75.
333. Roy, S., *Finding order in slow axonal transport.* Curr Opin Neurobiol, 2020. **63**: p. 87-94.
334. Reck-Peterson, S.L., et al., *The cytoplasmic dynein transport machinery and its many cargoes.* Nature Reviews Molecular Cell Biology, 2018. **19**(6): p. 382-398.
335. Mehta, A.D., et al., *Myosin-V is a processive actin-based motor.* Nature, 1999. **400**(6744): p. 590-3.
336. DePina, A.S. and G.M. Langford, *Vesicle transport: the role of actin filaments and myosin motors.* Microsc Res Tech, 1999. **47**(2): p. 93-106.
337. Coureux, P.D., et al., *A structural state of the myosin V motor without bound nucleotide.* Nature, 2003. **425**(6956): p. 419-23.
338. Coureux, P.D., H.L. Sweeney, and A. Houdusse, *Three myosin V structures delineate essential features of chemo-mechanical transduction.* EMBO J, 2004. **23**(23): p. 4527-37.
339. Trybus, K.M., *Myosin V from head to tail.* Cell Mol Life Sci, 2008. **65**(9): p. 1378-89.
340. Pathak, D., K.J. Sepp, and P.J. Hollenbeck, *Evidence that myosin activity opposes microtubule-based axonal transport of mitochondria.* J Neurosci, 2010. **30**(26): p. 8984-92.
341. Goldman, Y.E. and E.M. Ostap, *4.9 Myosin Motors: Kinetics of Myosin*, in *Comprehensive Biophysics*, E.H. Egelman, Editor. 2012, Elsevier: Amsterdam. p. 151-169.
342. Janssen, A.F.J., et al., *Myosin-V Induces Cargo Immobilization and Clustering at the Axon Initial Segment.* Frontiers in Cellular Neuroscience, 2017. **11**: p. 260.
343. Rind, H.B., R. Butowt, and C.S. von Bartheld, *Synaptic targeting of retrogradely transported trophic factors in motoneurons: comparison of glial cell line-derived neurotrophic factor, brain-derived neurotrophic factor, and cardiotrophin-1 with tetanus toxin.* J Neurosci, 2005. **25**(3): p. 539-49.
344. Chen, B.D., S. Hsu, and H.S. Lin, *Binding, internalization and degradation of colony-stimulating factor by peritoneal exudate macrophages.* Biochim Biophys Acta, 1982. **721**(4): p. 366-73.
345. Huang, J.S., et al., *Platelet-derived growth factor. Specific binding to target cells.* J Biol Chem, 1982. **257**(14): p. 8130-6.
346. Wakai, K., et al., *Processing of human growth hormone by rat hepatocytes in monolayer culture.* Endocrinology, 1984. **114**(5): p. 1475-82.
347. Davies, P.F., et al., *Metabolic cooperation between vascular endothelial cells and smooth muscle cells in co-culture: changes in low density lipoprotein metabolism.* J Cell Biol, 1985. **101**(3): p. 871-9.
348. Fujii, M., et al., *High-affinity receptor-mediated internalization and degradation of interleukin 2 in human T cells.* J Exp Med, 1986. **163**(3): p. 550-62.
349. Zoon, K.C., et al., *Analysis of the steady state binding, internalization, and degradation of human interferon-alpha2.* J Biol Chem, 1986. **261**(11): p. 4993-6.
350. Roupas, P. and A.C. Herington, *Receptor-mediated endocytosis and degradative processing of growth hormone by rat adipocytes in primary culture.* Endocrinology, 1987. **120**(5): p. 2158-65.
351. Sorkin, A., et al., *Multiple autophosphorylation site mutations of the epidermal growth factor receptor. Analysis of kinase activity and endocytosis.* J Biol Chem, 1991. **266**(13): p. 8355-62.

352. Yanai, S., et al., *Kinetic analysis of receptor-mediated endocytosis of epidermal growth factor by isolated rat hepatocytes.* Am J Physiol, 1991. **260**(3 Pt 1): p. C457-67.
353. Auletta, M., F.C. Nielsen, and S. Gammeltoft, *Receptor-mediated endocytosis and degradation of insulin-like growth factor I and II in neonatal rat astrocytes.* J Neurosci Res, 1992. **31**(1): p. 14-20.
354. Nielsen, S., *Time course and kinetics of proximal tubular processing of insulin.* Am J Physiol, 1992. **262**(5 Pt 2): p. F813-22.
355. Pandey, K.N., *Kinetic analysis of internalization, recycling and redistribution of atrial natriuretic factor-receptor complex in cultured vascular smooth-muscle cells. Ligand-dependent receptor down-regulation.* Biochem J, 1992. **288 (Pt 1)**(Pt 1): p. 55-61.
356. Zapf, A., D. Hsu, and J.M. Olefsky, *Comparison of the intracellular itineraries of insulin-like growth factor-I and insulin and their receptors in Rat-1 fibroblasts.* Endocrinology, 1994. **134**(6): p. 2445-52.
357. Pennica, D., et al., *Biochemical properties of the 75-kDa tumor necrosis factor receptor. Characterization of ligand binding, internalization, and receptor phosphorylation.* J Biol Chem, 1992. **267**(29): p. 21172-8.
358. Gleizes, P.E., et al., *Basic fibroblast growth factor (FGF-2) internalization through the heparan sulfate proteoglycans-mediated pathway: an ultrastructural approach.* Eur J Cell Biol, 1995. **66**(1): p. 47-59.
359. NCBI, G., *NGF nerve growth factor [Rattus norvegicus (Norway rat)]* 2018: GENE.
360. Campenot, R.B., *Regeneration of neurites on long-term cultures of sympathetic neurons deprived of nerve growth factor.* Science, 1981. **214**(4520): p. 579-81.
361. Seeley, P.J. and L.A. Greene, *Short-latency local actions of nerve growth factor at the growth cone.* Proc Natl Acad Sci U S A, 1983. **80**(9): p. 2789-93.
362. Martin, D.P., et al., *Inhibitors of protein synthesis and RNA synthesis prevent neuronal death caused by nerve growth factor deprivation.* J Cell Biol, 1988. **106**(3): p. 829-44.
363. Deckwerth, T.L. and E.M. Johnson, Jr., *Neurotrophic factor deprivation-induced death.* Ann N Y Acad Sci, 1993. **679**: p. 121-31.
364. Lanciego, J.L. and F.G. Wouterlood, *A half century of experimental neuroanatomical tracing.* J Chem Neuroanat, 2011. **42**(3): p. 157-83.
365. Campenot, R.B. and B.L. MacInnis, *Retrograde transport of neurotrophins: fact and function.* J Neurobiol, 2004. **58**(2): p. 217-29.
366. Sorbara, C.D., et al., *Pervasive axonal transport deficits in multiple sclerosis models.* Neuron, 2014. **84**(6): p. 1183-90.
367. van den Berg, R., C.C. Hoogenraad, and R.Q. Hintzen, *Axonal transport deficits in multiple sclerosis: spiraling into the abyss.* Acta Neuropathol, 2017. **134**(1): p. 1-14.
368. Guo, W., K. Stoklund Dittlau, and L. Van Den Bosch, *Axonal transport defects and neurodegeneration: Molecular mechanisms and therapeutic implications.* Semin Cell Dev Biol, 2020. **99**: p. 133-150.
369. Papasozomenos, S.C. and Y. Su, *Altered phosphorylation of tau protein in heat-shocked rats and patients with Alzheimer disease.* Proc Natl Acad Sci U S A, 1991. **88**(10): p. 4543-7.
370. Crutcher, K.A., et al., *Detection of NGF-like activity in human brain tissue: increased levels in Alzheimer's disease.* J Neurosci, 1993. **13**(6): p. 2540-50.
371. Goedert, M., et al., *Nerve growth factor receptor mRNA distribution in human brain: normal levels in basal forebrain in Alzheimer's disease.* Brain Res Mol Brain Res, 1989. **5**(1): p. 1-7.
372. Higgins, G.A. and E.J. Mufson, *NGF receptor gene expression is decreased in the nucleus basalis in Alzheimer's disease.* Exp Neurol, 1989b. **106**(3): p. 222-36.
373. Loy, R., T.A. Milner, and R.Y. Moore, *Sprouting of sympathetic axons in the hippocampal formation: conditions necessary to elicit ingrowth.* Exp Neurol, 1980. **67**(2): p. 399-41.
374. Phillips, H.S., et al., *BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease.* Neuron, 1991. **7**(5): p. 695-702.
375. Figlewicz, D.A., et al., *Variants of the heavy neurofilament subunit are associated with the development of amyotrophic lateral sclerosis.* Hum Mol Genet, 1994a. **3**(10): p. 1757-61.
376. Figlewicz, D.A., et al., *Identification of flanking markers for the familial amyotrophic lateral sclerosis gene ALS1 on chromosome 21.* J Neurol Sci, 1994b. **124 Suppl**: p. 90-5.
377. Collard, J.F., F. Côté, and J.P. Julien, *Defective axonal transport in a transgenic mouse model of amyotrophic lateral sclerosis.* Nature, 1995b. **375**(6526): p. 61-4.
378. Collard, J.F. and J.P. Julien, *A simple test to monitor the motor dysfunction in a transgenic mouse model of amyotrophic lateral sclerosis.* J Psychiatry Neurosci, 1995a. **20**(1): p. 80-2.

379. Bajaj, N.P., et al., *Alzheimer's disease, amyotrophic lateral sclerosis, and transgenic mice.* J Neurol Neurosurg Psychiatry, 1998. **64**(6): p. 711-5.
380. Tomkins, J., et al., *Novel insertion in the KSP region of the neurofilament heavy gene in amyotrophic lateral sclerosis (ALS).* Neuroreport, 1998. **9**(17): p. 3967-70.
381. Al-Chalabi, A., et al., *Deletions of the heavy neurofilament subunit tail in amyotrophic lateral sclerosis.* Hum Mol Genet, 1999. **8**(2): p. 157-64.
382. Gros-Louis, F., et al., *A frameshift deletion in peripherin gene associated with amyotrophic lateral sclerosis.* J Biol Chem, 2004. **279**(44): p. 45951-6.
383. Ackerley, S., et al., *p38alpha stress-activated protein kinase phosphorylates neurofilaments and is associated with neurofilament pathology in amyotrophic lateral sclerosis.* Mol Cell Neurosci, 2004. **26**(2): p. 354-64.
384. Millecamps, S., et al., *Defective axonal transport of neurofilament proteins in neurons overexpressing peripherin.* J Neurochem, 2006. **98**(3): p. 926-38.
385. De Vos, K.J., et al., *Role of axonal transport in neurodegenerative diseases.* Annu Rev Neurosci, 2008. **31**: p. 151-73.
386. Oberstadt, M., et al., *TDP-43 and Cytoskeletal Proteins in ALS.* Mol Neurobiol, 2018. **55**(4): p. 3143-3151.
387. Hergesheimer, R.C., et al., *The debated toxic role of aggregated TDP-43 in amyotrophic lateral sclerosis: a resolution in sight?* Brain, 2019. **142**(5): p. 1176-1194.
388. Hergesheimer, R., et al., *Advances in disease-modifying pharmacotherapies for the treatment of amyotrophic lateral sclerosis.* Expert Opin Pharmacother, 2020. **21**(9): p. 1103-1110.
389. Volkening, K., et al., *Tar DNA binding protein of 43 kDa (TDP-43), 14-3-3 proteins and copper/zinc superoxide dismutase (SOD1) interact to modulate NFL mRNA stability. Implications for altered RNA processing in amyotrophic lateral sclerosis (ALS).* Brain Res, 2009. **1305**: p. 168-82.
390. Prasad, A., et al., *Molecular Mechanisms of TDP-43 Misfolding and Pathology in Amyotrophic Lateral Sclerosis.* Front Mol Neurosci, 2019. **12**: p. 25.
391. Alami, N.H., et al., *Axonal transport of TDP-43 mRNA granules is impaired by ALS-causing mutations.* Neuron, 2014. **81**(3): p. 536-543.
392. Smith, A.L., et al., *Amyotrophic lateral sclerosis in a patient with a family history of huntington disease: genetic counseling challenges.* J Genet Couns, 2014. **23**(5): p. 725-33.
393. Loeffler, T., et al., *Neurofilament-Light Chain as Biomarker of Neurodegenerative and Rare Diseases With High Translational Value.* Front Neurosci, 2020. **14**: p. 579.
394. Fédou, C., et al., *The low affinity p75 neurotrophin receptor is down-regulated in congenital anomalies of the kidney and the urinary tract: Possible involvement in early nephrogenesis.* Biochem Biophys Res Commun, 2020. **533**(4): p. 786-791.
395. Brownlees, J., et al., *Charcot-Marie-Tooth disease neurofilament mutations disrupt neurofilament assembly and axonal transport.* Hum Mol Genet, 2002. **11**(23): p. 2837-44.
396. Jordanova, A., et al., *Mutations in the neurofilament light chain gene (NEFL) cause early onset severe Charcot-Marie-Tooth disease.* Brain, 2003a. **126**(Pt 3): p. 590-7.
397. Jordanova, A., et al., *Dominant intermediate Charcot-Marie-Tooth type C maps to chromosome 1p34-p35.* Am J Hum Genet, 2003b. **73**(6): p. 1423-30.
398. Züchner, S. and J.M. Vance, *Mechanisms of disease: a molecular genetic update on hereditary axonal neuropathies.* Nat Clin Pract Neurol, 2006a. **2**(1): p. 45-53.
399. Züchner, S. and J.M. Vance, *Molecular genetics of autosomal-dominant axonal Charcot-Marie-Tooth disease.* Neuromolecular Med, 2006b. **8**(1-2): p. 63-74.
400. Lancaster, E., et al., *Myelinated axons fail to develop properly in a genetically authentic mouse model of Charcot-Marie-Tooth disease type 2E.* Exp Neurol, 2018. **308**: p. 13-25.
401. Roll-Mecak, A. and R.D. Vale, *Structural basis of microtubule severing by the hereditary spastic paraplegia protein spastin.* Nature, 2008. **451**(7176): p. 363-7.
402. Dion, P.A., H. Daoud, and G.A. Rouleau, *Genetics of motor neuron disorders: new insights into pathogenic mechanisms.* Nat Rev Genet, 2009. **10**(11): p. 769-82.
403. Shribman, S., et al., *Hereditary spastic paraplegia: from diagnosis to emerging therapeutic approaches.* Lancet Neurol, 2019. **18**(12): p. 1136-1146.
404. Berth, S.H., et al., *HIV Glycoprotein Gp120 Impairs Fast Axonal Transport by Activating Tak1 Signaling Pathways.* ASN Neuro, 2016. **8**(6).

405. Bracci-Laudiero, L., et al., *CD34-positive cells in human umbilical cord blood express nerve growth factor and its specific receptor TrkA.* J Neuroimmunol, 2003. **136**(1-2): p. 130-9.
406. Prencipe, G., et al., *Nerve growth factor downregulates inflammatory response in human monocytes through TrkA.* J Immunol, 2014. **192**(7): p. 3345-54.
407. Motil, J., et al., *Dynein mediates retrograde neurofilament transport within axons and anterograde delivery of NFs from perikarya into axons: regulation by multiple phosphorylation events.* Cell Motil Cytoskeleton, 2006. **63**(5): p. 266-86.
408. Liu, J.J., et al., *Retrolinkin, a membrane protein, plays an important role in retrograde axonal transport.* Proc Natl Acad Sci U S A, 2007. **104**(7): p. 2223-8.
409. Perlson, E., et al., *A switch in retrograde signaling from survival to stress in rapid-onset neurodegeneration.* J Neurosci, 2009. **29**(31): p. 9903-17.
410. Liu, X.A., V. Rizzo, and S.V. Puthanveettil, *PATHOLOGIES OF AXONAL TRANSPORT IN NEURODEGENERATIVE DISEASES.* Transl Neurosci, 2012. **3**(4): p. 355-372.
411. Smith, R.B., et al., *Relay of retrograde synaptogenic signals through axonal transport of BMP receptors.* J Cell Sci, 2012. **125**(Pt 16): p. 3752-64.
412. Yang, P., et al., *VEGF Axonal Transport Dependent on Kinesin-1B and Microtubules Dynamics.* Frontiers in Molecular Neuroscience, 2017. **10**: p. 424.
413. Geffen, L.B. and B.G. Livett, *Synaptic vesicles in sympathetic neurons.* Physiol Rev, 1971. **51**(1): p. 98-157.
414. Ochs, S., *Characteristics and a model for fast axoplasmic transport in nerve.* J Neurobiol, 1971. **2**(4): p. 331-45.
415. Banks, P. and D. Mayor, *Axoplasmic transport in postganglionic sympathetic neurons.* Biochem J, 1972. **128**(3): p. 77p-78p.
416. Lindqvist, N., et al., *Multiple receptor tyrosine kinases are expressed in adult rat retinal ganglion cells as revealed by single-cell degenerate primer polymerase chain reaction.* Ups J Med Sci, 2010. **115**(1): p. 65-80.
417. Carmignoto, G., et al., *Effect of NGF on the survival of rat retinal ganglion cells following optic nerve section.* J Neurosci, 1989. **9**(4): p. 1263-72.
418. Frade, J.M., A. Rodríguez-Tébar, and Y.A. Barde, *Induction of cell death by endogenous nerve growth factor through its p75 receptor.* Nature, 1996. **383**(6596): p. 166-8.
419. Frade, J.M., P. Bovolenta, and A. Rodriguez-Tebar, *Neurotrophins and other growth factors in the generation of retinal neurons.* Microsc Res Tech, 1999. **45**(4-5): p. 243-51.
420. Lambiase, A., et al., *Expression of nerve growth factor receptors on the ocular surface in healthy subjects and during manifestation of inflammatory diseases.* Invest Ophthalmol Vis Sci, 1998. **39**(7): p. 1272-5.
421. Lambiase, A., et al., *Nerve growth factor promotes corneal healing: structural, biochemical, and molecular analyses of rat and human corneas.* Invest Ophthalmol Vis Sci, 2000. **41**(5): p. 1063-9.
422. Willard, M.B. and K.L. Hulebak, *The intra-axonal transport of polypeptide H: evidence for a fifth (very slow) group of transported proteins in the retinal ganglion cells of the rabbit.* Brain Res, 1977. **136**(2): p. 289-306.
423. Levine, J. and M. Willard, *The composition and organization of axonally transported proteins in the retinal ganglion cells of the guinea pig.* Brain Res, 1980. **194**(1): p. 137-54.
424. McQuarrie, I.G., S.T. Brady, and R.J. Lasek, *Diversity in the axonal transport of structural proteins: major differences between optic and spinal axons in the rat.* J Neurosci, 1986. **6**(6): p. 1593-605.
425. Forman, D.S., A.L. Padjen, and G.R. Siggins, *Effect of temperature on the rapid retrograde transport of microscopically visible intra-axonal organelles.* Brain Res, 1977. **136**(2): p. 215-26.
426. Forman, D.S., A.L. Padjen, and G.R. Siggins, *Axonal transport of organelles visualized by light microscopy: cinemicrographic and computer analysis.* Brain Res, 1977b. **136**(2): p. 197-213.
427. Koles, Z.J., K.D. McLeod, and R.S. Smith, *A study of the motion of organelles which undergo retrograde and anterograde rapid axonal transport in Xenopus.* J Physiol, 1982. **328**: p. 469-84.
428. Breuer, A.C., et al., *Fast axonal transport in amyotrophic lateral sclerosis: an intra-axonal organelle traffic analysis.* Neurology, 1987. **37**(5): p. 738-48.
429. Abbate, S.L., M.B. Atkinson, and A.C. Breuer, *Amount and speed of fast axonal transport in diabetes.* Diabetes, 1991. **40**(1): p. 111-7.
430. Le Bivic, A., et al., *An internal deletion in the cytoplasmic tail reverses the apical localization of human NGF receptor in transfected MDCK cells.* J Cell Biol, 1991. **115**(3): p. 607-18.

431. Kahle, P. and C. Hertel, *Nerve growth factor (NGF) receptor on rat glial cell lines. Evidence for NGF internalization via p75NGFR.* J Biol Chem, 1992. **267**(20): p. 13917-23.
432. Kahle, P., et al., *p75 nerve growth factor receptor modulates p140trkA kinase activity, but not ligand internalization, in PC12 cells.* J Neurosci Res, 1994. **38**(5): p. 599-606.
433. Mahadeo, D., et al., *High affinity nerve growth factor binding displays a faster rate of association than p140trk binding. Implications for multi-subunit polypeptide receptors.* J Biol Chem, 1994. **269**(9): p. 6884-91.
434. Bibel, M., E. Hoppe, and Y.A. Barde, *Biochemical and functional interactions between the neurotrophin receptors trk and p75NTR.* Embo j, 1999. **18**(3): p. 616-22.
435. He, X.L. and K.C. Garcia, *Structure of nerve growth factor complexed with the shared neurotrophin receptor p75.* Science, 2004. **304**(5672): p. 870-5.
436. Barker, P.A., *p75NTR is positively promiscuous: novel partners and new insights.* Neuron, 2004. **42**(4): p. 529-33.
437. Dechant, G. and Y.A. Barde, *The neurotrophin receptor p75(NTR): novel functions and implications for diseases of the nervous system.* Nat Neurosci, 2002. **5**(11): p. 1131-6.
438. Ibáñez, C.F. and A. Simi, *p75 neurotrophin receptor signaling in nervous system injury and degeneration: paradox and opportunity.* Trends Neurosci, 2012. **35**(7): p. 431-40.
439. Lee, R., et al., *Regulation of cell survival by secreted proneurotrophins.* Science, 2001. **294**(5548): p. 1945-8.
440. Nykjaer, A., et al., *Sortilin is essential for proNGF-induced neuronal cell death.* Nature, 2004. **427**(6977): p. 843-8.
441. Barrett, G.L., et al., *Co-expression of the P75 neurotrophin receptor and neurotrophin receptor-interacting melanoma antigen homolog in the mature rat brain.* Neuroscience, 2005. **133**(2): p. 381-92.
442. Underwood, C.K. and E.J. Coulson, *The p75 neurotrophin receptor.* Int J Biochem Cell Biol, 2008. **40**(9): p. 1664-8.
443. Pham, D.D., et al., *Caspase-2 and p75 neurotrophin receptor (p75NTR) are involved in the regulation of SREBP and lipid genes in hepatocyte cells.* Cell Death Dis, 2019. **10**(7): p. 537.
444. Melemedjian, O.K., et al., *Local translation and retrograde axonal transport of CREB regulates IL-6-induced nociceptive plasticity.* Mol Pain, 2014. **10**: p. 45.
445. Mar, F.M., et al., *CNS axons globally increase axonal transport after peripheral conditioning.* J Neurosci, 2014. **34**(17): p. 5965-70.
446. Moy, J.K., et al., *eIF4E Phosphorylation Influences Bdnf mRNA Translation in Mouse Dorsal Root Ganglion Neurons.* Front Cell Neurosci, 2018. **12**: p. 29.
447. May, P.J., *Superior Colliculus*, in *Encyclopedia of the Neurological Sciences (Second Edition)*, M.J. Aminoff and R.B. Daroff, Editors. 2014, Academic Press: Oxford. p. 351-354.
448. Enochs, W.S., et al., *MR imaging of slow axonal transport in vivo.* Exp Neurol, 1993. **123**(2): p. 235-42.
449. Greene, L.A. and A.S. Tischler, *Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor.* Proc Natl Acad Sci U S A, 1976. **73**(7): p. 2424-8.
450. Greene, L.A. and G. Rein, *Release, storage and uptake of catecholamines by a clonal cell line of nerve growth factor (NGF) responsive pheo-chromocytoma cells.* Brain Res, 1977. **129**(2): p. 247-63.
451. Wehrman, T., et al., *Structural and mechanistic insights into nerve growth factor interactions with the TrkA and p75 receptors.* Neuron, 2007. **53**(1): p. 25-38.
452. Gong, Y., et al., *Crystal structure of the neurotrophin-3 and p75NTR symmetrical complex.* Nature, 2008. **454**(7205): p. 789-93.
453. Masoudi, R., et al., *Biological activity of nerve growth factor precursor is dependent upon relative levels of its receptors.* J Biol Chem, 2009. **284**(27): p. 18424-33.
454. Lamballe, F., R. Klein, and M. Barbacid, *The trk family of oncogenes and neurotrophin receptors.* Princess Takamatsu Symp, 1991b. **22**: p. 153-70.
455. Kummar, S. and U.N. Lassen, *TRK Inhibition: A New Tumor-Agnostic Treatment Strategy.* Targeted Oncology, 2018. **13**(5): p. 545-556.
456. Amatu, A., et al., *Tropomyosin receptor kinase (TRK) biology and the role of NTRK gene fusions in cancer.* Ann Oncol, 2019. **30**(Suppl_8): p. viii5-viii15.
457. Milione, M., et al., *Identification and characterization of a novel SCYL3-NTRK1 rearrangement in a colorectal cancer patient.* Oncotarget, 2017. **8**(33): p. 55353-55360.

458. Jang, S.W., et al., *Amitriptyline is a TrkA and TrkB receptor agonist that promotes TrkA/TrkB heterodimerization and has potent neurotrophic activity.* Chem Biol, 2009. **16**(6): p. 644-56.
459. Jang, S.W., et al., *Gambogic amide, a selective agonist for TrkA receptor that possesses robust neurotrophic activity, prevents neuronal cell death.* Proc Natl Acad Sci U S A, 2007. **104**(41): p. 16329-34.
460. Zheng, X., et al., *Amitriptyline Activates TrkA to Aid Neuronal Growth and Attenuate Anesthesia-Induced Neurodegeneration in Rat Dorsal Root Ganglion Neurons.* Medicine (Baltimore), 2016. **95**(18): p. e3559.
461. Cocco, E., M. Scaltriti, and A. Drilon, *NTRK fusion-positive cancers and TRK inhibitor therapy.* Nat Rev Clin Oncol, 2018. **15**(12): p. 731-747.
462. Drilon, A., *TRK inhibitors in TRK fusion-positive cancers.* Ann Oncol, 2019. **30**(Suppl_8): p. viii23-viii30.
463. Weintraub, E., *Eli Lilly grabs spotlight at J.P. Morgan with $8B Loxo Oncology buyout* in *FIERCE PHARMA*. 2019: Online.
464. FDA, *FDA approves larotrectinib for solid tumors with NTRK gene fusions*. 2018.
465. VIKTRAVI, *Viktravi (larotrectinib) prescribing information*. 2018, Bayer HealthCare Pharmaceuticals Inc.: Online.
466. FDA, *FDA approves first drug for neurotrophic keratitis, a rare eye disease*. 2018: online.
467. Roberti, G., et al., *Nerve growth factor modulation of retinal ganglion cell physiology.* J Cell Physiol, 2014. **229**(9): p. 1130-3.
468. Bonini, S., et al., *Phase I Trial of Recombinant Human Nerve Growth Factor for Neurotrophic Keratitis.* Ophthalmology, 2018a. **125**(9): p. 1468-1471.
469. Bonini, S., et al., *Phase II Randomized, Double-Masked, Vehicle-Controlled Trial of Recombinant Human Nerve Growth Factor for Neurotrophic Keratitis.* Ophthalmology, 2018b. **125**(9): p. 1332-1343.
470. Bhattacharyya, A., et al., *Trk receptors function as rapid retrograde signal carriers in the adult nervous system.* J Neurosci, 1997. **17**(18): p. 7007-16.
471. Wang, H., et al., *The nerve growth factor signaling and its potential as therapeutic target for glaucoma.* Biomed Res Int, 2014. **2014**: p. 759473.
472. Munch, M., B.H. Rotstein, and G. Ulrich, *Fluorine-18-Labeled Fluorescent Dyes for Dual-Mode Molecular Imaging.* Molecules, 2020. **25**(24).
473. Koch, M., et al., *Threshold Analysis and Biodistribution of Fluorescently Labeled Bevacizumab in Human Breast Cancer.* Cancer Res, 2017. **77**(3): p. 623-631.
474. Lamberts, L.E., et al., *Tumor-Specific Uptake of Fluorescent Bevacizumab–IRDye800CW Microdosing in Patients with Primary Breast Cancer: A Phase I Feasibility Study.* Clinical Cancer Research, 2017. **23**(11): p. 2730.
475. Gao, R.W., et al., *Determination of Tumor Margins with Surgical Specimen Mapping Using Near-Infrared Fluorescence.* Cancer Res, 2018. **78**(17): p. 5144-5154.
476. Nishio, N., et al., *Optimal Dosing Strategy for Fluorescence-Guided Surgery with Panitumumab-IRDye800CW in Head and Neck Cancer.* Mol Imaging Biol, 2020. **22**(1): p. 156-164.
477. Sorger, J., *PHARMACEUTICAL COMPOSITIONS OF NEAR IR CLOSED CHAIN, SULFO-CYANINE DYES*. 2019, Intuitive Surgical Operations, Inc. (Sunnyvale, CA): US.
478. Clinicaltrials.gov, *A Safety and Efficacy Study of IS-001 Injection in Patients Undergoing Robotic Hysterectomy*, I. Sponsor Intuitive Surgical, Editor. 2021: www.clinicaltrials.gov.
479. Farnam, R.W., et al., *Intraoperative ureter visualization using a near-infrared imaging agent.* J Biomed Opt, 2019. **24**(6): p. 1-8.
480. Parada, L.F., et al., *The Trk family of tyrosine kinases: receptors for NGF-related neurotrophins.* Cold Spring Harb Symp Quant Biol, 1992. **57**: p. 43-51.
481. Reichardt, L.F., *Neurotrophin-regulated signalling pathways.* Philos Trans R Soc Lond B Biol Sci, 2006. **361**(1473): p. 1545-64.
482. Sorkin, A. and M. von Zastrow, *Endocytosis and signalling: intertwining molecular networks.* Nat Rev Mol Cell Biol, 2009. **10**(9): p. 609-22.
483. Yamashita, N., *Retrograde signaling via axonal transport through signaling endosomes.* J Pharmacol Sci, 2019. **141**(2): p. 91-96.
484. Leibrock, J., et al., *Molecular cloning and expression of brain-derived neurotrophic factor.* Nature, 1989. **341**(6238): p. 149-52.
485. Jones, K.R. and L.F. Reichardt, *Molecular cloning of a human gene that is a member of the nerve growth factor family.* Proc Natl Acad Sci U S A, 1990. **87**(20): p. 8060-4.

486. Levi-Montalcini, R., *THE NERVE GROWTH FACTOR.* Ann N Y Acad Sci, 1964. **118**: p. 149-70.
487. Angeletti, P.U., et al., *EFFECT OF A NERVE GROWTH FACTOR ON GLUCOSE METABOLISM BY SYMPATHETIC AND SENSORY NERVE CELLS.* Biochim Biophys Acta, 1964. **90**: p. 445-50.
488. Levi-Montalcini, R. and P.U. Angeletti, *Nerve growth factor.* Physiol Rev, 1968. **48**(3): p. 534-69.
489. Levi-Montalcini, R., et al., *In vitro effects of the nerve growth factor on the fine structure of the sensory nerve cells.* Brain Res, 1968c. **8**(2): p. 347-62.
490. Angeletti, P.U., R. Levi-Montalcini, and P. Calissano, *The nerve growth factor (NGF): chemical properties and metabolic effects.* Adv Enzymol Relat Areas Mol Biol, 1968. **31**: p. 51-75.
491. Zanini, A., P. Angeletti, and R. Levi-Montalcini, *Immunochemical properties of the nerve growth factor.* Proc Natl Acad Sci U S A, 1968. **61**(3): p. 835-42.
492. Li, Y., et al., *Tropomyosin receptor kinase B (TrkB) signalling: targeted therapy in neurogenic tumours.* J Pathol Clin Res, 2023. **9**(2): p. 89-99.
493. Ibanez, C.F., et al., *An extended surface of binding to Trk tyrosine kinase receptors in NGF and BDNF allows the engineering of a multifunctional pan-neurotrophin.* Embo j, 1993. **12**(6): p. 2281-93.
494. Kashiba, H., et al., *Coexpression of trk family members and low-affinity neurotrophin receptors in rat dorsal root ganglion neurons.* Brain Res Mol Brain Res, 1995. **30**(1): p. 158-64.
495. Yamamoto, M., et al., *Expression of mRNAs for neurotrophic factors (NGF, BDNF, NT-3, and GDNF) and their receptors (p75NGFR, trkA, trkB, and trkC) in the adult human peripheral nervous system and nonneural tissues.* Neurochem Res, 1996. **21**(8): p. 929-38.
496. Sleigh, J.N., A.P. Tosolini, and G. Schiavo, *In Vivo Imaging of Anterograde and Retrograde Axonal Transport in Rodent Peripheral Nerves*, in *Methods Mol Biol*, A.D.M.a.P. Elisabetta Babetto (ed.), Methods in Molecular Biology, vol. 2143, and T.A.s. https://doi.org/10.1007/978-1-0716-0585-1_20, Editors. 2020. p. 271-292.
497. Ahn, S., A. Riccio, and D.D. Ginty, *Spatial considerations for stimulus-dependent transcription in neurons.* Annu Rev Physiol, 2000. **62**: p. 803-23.
498. Ginty, D.D. and R.A. Segal, *Retrograde neurotrophin signaling: Trk-ing along the axon.* Curr Opin Neurobiol, 2002. **12**(3): p. 268-74.
499. Banfield, M.J., et al., *Specificity in Trk receptor:neurotrophin interactions: the crystal structure of TrkB-d5 in complex with neurotrophin-4/5.* Structure, 2001. **9**(12): p. 1191-9.
500. Robinson, R.C., et al., *Structure of the brain-derived neurotrophic factor/neurotrophin 3 heterodimer.* Biochemistry, 1995. **34**(13): p. 4139-46.
501. Kalinowska-Lyszczarz, A. and J. Losy, *The role of neurotrophins in multiple sclerosis-pathological and clinical implications.* Int J Mol Sci, 2012. **13**(10): p. 13713-25.
502. Barbacid, M., *Neurotrophic factors and their receptors.* Curr Opin Cell Biol, 1995a. **7**(2): p. 148-55.
503. Ibáñez, C.F., G. Paratcha, and F. Ledda, *RET-independent signaling by GDNF ligands and GFRα receptors.* Cell Tissue Res, 2020. **382**(1): p. 71-82.
504. Takahashi, M., J. Ritz, and G.M. Cooper, *Activation of a novel human transforming gene, ret, by DNA rearrangement.* Cell, 1985. **42**(2): p. 581-8.
505. Rose-John, S., *Interleukin-6 Family Cytokines.* Cold Spring Harb Perspect Biol, 2018. **10**(2).
506. Ferrari, M.P., et al., *Safety and pharmacokinetics of escalating doses of human recombinant nerve growth factor eye drops in a double-masked, randomized clinical trial.* BioDrugs, 2014. **28**(3): p. 275-83.
507. Chang, H.M., et al., *Neurotrophins and glial cell line-derived neurotrophic factor in the ovary: physiological and pathophysiological implications.* Hum Reprod Update, 2019.
508. Skaper, S.D., *Neurotrophic Factors: An Overview.* Methods Mol Biol, 2018. **1727**: p. 1-17.
509. Richardson, P.M., *Ciliary neurotrophic factor: a review.* Pharmacol Ther, 1994. **63**(2): p. 187-98.
510. McDonald, N.Q., N. Panayotatos, and W.A. Hendrickson, *Crystal structure of dimeric human ciliary neurotrophic factor determined by MAD phasing.* Embo j, 1995. **14**(12): p. 2689-99.
511. Eigenbrot, C. and N. Gerber, *X-ray structure of glial cell-derived neurotrophic factor at 1.9 A resolution and implications for receptor binding.* Nat Struct Biol, 1997. **4**(6): p. 435-8.
512. Treanor, J.J., et al., *Characterization of a multicomponent receptor for GDNF.* Nature, 1996. **382**(6586): p. 80-3.
513. Takaso, Y., et al., *Deletion of CD38 and supplementation of NAD(+) attenuate axon degeneration in a mouse facial nerve axotomy model.* Sci Rep, 2020. **10**(1): p. 17795.
514. Jimenez-Andrade, J.M., et al., *Pathological sprouting of adult nociceptors in chronic prostate cancer-induced bone pain.* J Neurosci, 2010. **30**(44): p. 14649-56.

515. Castaneda-Corral, G., et al., *The majority of myelinated and unmyelinated sensory nerve fibers that innervate bone express the tropomyosin receptor kinase A.* Neuroscience, 2011. **178**: p. 196-207.
516. Bennett, D.L., et al., *Endogenous nerve growth factor regulates the sensitivity of nociceptors in the adult rat.* Eur J Neurosci, 1998. **10**(4): p. 1282-91.
517. Spillane, M., et al., *Nerve growth factor-induced formation of axonal filopodia and collateral branches involves the intra-axonal synthesis of regulators of the actin-nucleating Arp2/3 complex.* J Neurosci, 2012. **32**(49): p. 17671-89.
518. Levi-Montalcini, R. and B. Booker, *EXCESSIVE GROWTH OF THE SYMPATHETIC GANGLIA EVOKED BY A PROTEIN ISOLATED FROM MOUSE SALIVARY GLANDS.* Proc Natl Acad Sci U S A, 1960. **46**(3): p. 373-84.
519. Torcia, M., et al., *Nerve growth factor is an autocrine survival factor for memory B lymphocytes.* Cell, 1996. **85**(3): p. 345-56.
520. Bracci-Laudiero, L., *NGF and inflammation: a complex bidirectional interaction. .* 2010.
521. Bracci-Laudiero, L., et al., *NGF modulates CGRP synthesis in human B-lymphocytes: a possible anti-inflammatory action of NGF?* J Neuroimmunol, 2002. **123**(1-2): p. 58-65.
522. Bracci-Laudiero, L., et al., *Increased levels of NGF in sera of systemic lupus erythematosus patients.* Neuroreport, 1993. **4**(5): p. 563-5.
523. Mason, A. and K.J. Muller, *Axon segments sprout at both ends: tracking growth with fluorescent D-peptides.* Nature, 1982. **296**(5858): p. 655-7.
524. Nascimento, F.P., et al., *Sympathetic fibre sprouting in the skin contributes to pain-related behaviour in spared nerve injury and cuff models of neuropathic pain.* Mol Pain, 2015. **11**: p. 59.
525. Keefe, K.M., I.S. Sheikh, and G.M. Smith, *Targeting Neurotrophins to Specific Populations of Neurons: NGF, BDNF, and NT-3 and Their Relevance for Treatment of Spinal Cord Injury.* Int J Mol Sci, 2017. **18**(3).
526. Myckatyn, T.M. and S.E. Mackinnon, *A review of facial nerve anatomy.* Semin Plast Surg, 2004. **18**(1): p. 5-12.
527. Huang, H., et al., *PI3K/Akt and ERK/MAPK Signaling Promote Different Aspects of Neuron Survival and Axonal Regrowth Following Rat Facial Nerve Axotomy.* Neurochem Res, 2017. **42**(12): p. 3515-3524.
528. Akazawa, C., et al., *The upregulated expression of sonic hedgehog in motor neurons after rat facial nerve axotomy.* J Neurosci, 2004. **24**(36): p. 7923-30.
529. Niemi, J.P., et al., *A critical role for macrophages near axotomized neuronal cell bodies in stimulating nerve regeneration.* J Neurosci, 2013. **33**(41): p. 16236-48.
530. Sendtner, M., G.W. Kreutzberg, and H. Thoenen, *Ciliary neurotrophic factor prevents the degeneration of motor neurons after axotomy.* Nature, 1990. **345**(6274): p. 440-1.
531. Goodness, T.P., et al., *Overexpression of nerve growth factor in skin increases sensory neuron size and modulates Trk receptor expression.* Eur J Neurosci, 1997. **9**(8): p. 1574-85.
532. Petruska, J.C. and L.M. Mendell, *The many functions of nerve growth factor: multiple actions on nociceptors.* Neurosci Lett, 2004. **361**(1-3): p. 168-71.
533. Huang, E.J., et al., *Expression of Trk receptors in the developing mouse trigeminal ganglion: in vivo evidence for NT-3 activation of TrkA and TrkB in addition to TrkC.* Development, 1999. **126**(10): p. 2191-203.