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(54) Title: METHODS FOR TREATING AMYOTROPHIC LATERAL SCLEROSIS WITH CNTF

(57) Abstract

A method is provided for the treatment of ALS which is comprised of administering a therapeutically effective amount of ciliary neurotrophic factor (CNTF).

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METHODS FOR TREATING AMYOTROPHIC LATERAL SCLEROSIS WITH CNTF

BACKGROUND OF THE INVENTION

The present invention includes methods for the treatment of amyotrophic lateral sclerosis (ALS) in human patients.

Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disorder of motor neurons in the spinal cord, brainstem, and motor cortex, manifested clinically by muscular weakness, atrophy, and corticospinal tract signs in varying combinations.

ALS is a common disease, with an annual incidence rate of 0.4 to 1.76 per 100,000 population (approximately 1,000-4,300 in the U.S.). Most patients are more than 50 years old at the onset of symptoms and the incidence increases with each decade of life. ALS occurs in a random pattern throughout the world; it is estimated that in about 5% of cases ALS is familial, being inherited as an autosomal dominant trait.

ALS affects neuromuscular functions in the hand, leg, thoracic, abdominal, or posterior neck muscles. Typical initial symptoms in the hand include uselessness of hand, awkwardness in tasks requiring fine finger movements, stiffness of the fingers, and slight weakness or wasting of the hand muscles. Cramping and fasciculations of the muscles of the forearm, upper arm, and shoulder girdle muscles also appears. With time, the other hand and arm may be similarly affected. Eventually a patient exhibits atrophic weakness of the hands and forearms, slight spasticity of the legs, and generalized hyperreflexia. Muscle strength and bulk diminish, abductors, adductors, and extensors of fingers and thumb tend to become weak before the long flexors, on which the handgrip depends, and the dorsal interosseous spaces become hollowed, giving rise to the "cadaveric" or "skeleton hand". The patient may walk about with

useless, dangling arms. Later the atrophic weakness spreads to the neck, tongue, pharyngeal and laryngeal muscles.

The principal finding is a loss of nerve cells in the anterior horns of the spinal cord and motor nuclei of the lower brainstem. There is an extensive neuronal loss and gliosis involving the premotor area, particularly the superior frontal gyri, and the inferolateral cortex of the temporal lobes. Many of the surviving nerve cells are small, shrunken, and filled with lipofuscin. Lost cells are replaced by fibrous astrocytes.

ALS has been observed in conjunction with presenile and senile dementia, and with Parkinsons disease. The course of ALS, irrespective of its particular mode of onset and pattern of evolution, is inexorably progressive. Half of the patients die within 3 years and 90% within 6 years (Adams and Victor (1989) in Principles of Neurology, 4th ed., McGraw-Hill, Inc., New York.)

In diseases which cause extensive damage to striated muscle fibers, intracellular muscle fiber enzymes leak out of the cell and enter the bloodstream. One of the most commonly measured enzymes, and one of the most sensitive measures of muscle damage, is creatine phosphokinase (CPK). Serum CPK levels are used to monitor muscle damage in certain neuromuscular diseases, and as a marker for effective treatment. In disease which cause denervation paralysis with muscular atrophy, such as ALS, serum CPK levels may be elevated up to 2-3 times normal. This phenomenon, occurring in almost half of all patients with ALS, probably represents ongoing muscle damage secondary to progressive denervation.

A standardized test has been developed to measure the clinical deficit and progression of ALS. The test battery, developed by the Neuromuscular Research Unit

at the New England Medical Center is referred to as the Tufts Quantitative Neuromuscular Exam or TQNE. See, Andres et al. Neurology 38: 409-413 (1988). The TQNE is a validated test battery specifically designed to measure the motor function and strength of ALS patients as the disease progresses. The battery includes testing of five major functional areas: bulbar, respiratory, arm, leg, and fine motor activities.

The present invention includes the use of the protein neurotrophic factor ciliary neurotrophic factor (CNTF) to treat ALS. Neurotrophic factors are naturally occurring proteins that promote the survival and functional activities of nerve cells. Neurotrophic factors have been found in the target cells to which an innervating nerve cell connects. Such target-derived neurotrophic factors regulate the number of contacts formed between innervating nerve cells and the target cell population, and are necessary for the survival and maintenance of these nerve cells.

Neurotrophic factors are also found in cells that are not innervated. An example of such a neurotrophic factor is CNTF. Human CNTF and the gene encoding human CNTF are described in detail in United States patent numbers 4,997,929, 5,141,856 and co-pending United States patent application serial number 07/857,544 filed March 24, 1992. Each of these documents are specifically incorporated herein by this reference. Although the biological role of CNTF has not been conclusively established, CNTF appears to be released upon injury to the nervous system and may limit the extent of injury or neuronal damage.

Highly-purified CNTF has been shown to support the survival in cell cultures of chick embryonic parasympathetic, sympathetic, sensory, and motor neurons. There is significant evidence to support the proposition that CNTF is a neurotrophic factor for peripheral primary neurons in vivo and in vitro. See,

U.S. patent application serial number 07/735,538 filed July 23, 1991, specifically incorporated herein by this reference.

In the present invention, methods are provided for the treatment of ALS by the administration of ciliary neurotrophic factor (CNTF).

SUMMARY OF THE INVENTION

The present invention includes methods for the treatment of ALS by administering a human protein ciliary neurotrophic factor to a patient in need thereof. In particular, the invention provides methods for administering therapeutically effective amounts of CNTF by therapeutically effective routes of administration in order to treat patients suffering from ALS.

In one preferred embodiment of the invention, recombinant human CNTF is administered to human patients suffering from ALS in doses of less than about 10 μg/kg/day. More specifically, daily doses of about 2-3 μg/kg/day are utilized.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Reference will now be made in detail to the presently preferred embodiments of the invention, which, together with the following examples, serve to explain the principles of the invention.

The present invention includes a method for treating a patient suffering from ALS by administering to that patient the human neurotrophic factor CNTF.

In one embodiment of this invention, preferred CNTFs are naturally occurring proteins. The naturally-occurring proteins are preferred in part because they

pose a possibly lower risk of producing unforeseen and undesirable physiological side effects in patients treated therewith. Human CNTFs are preferred for use in this invention. However, to the extent that non-human CNTFs are substantially equivalent to human CNTFs and possess equivalent biological activity, they are considered to be within the scope of this invention.

For purposes of the specification and claims, a protein is deemed to be "naturally-occurring" if it or a substantially equivalent protein can be found to exist normally in healthy humans. "Naturally-occurring" proteins specifically includes forms of proteins found to exist in healthy humans that are partially truncated at the amino or carboxyl terminus of such proteins or that have amino acids that are deamidated or otherwise chemically modified. "Naturally-occurring" proteins may be obtained by recombinant DNA methods as well as by isolation from cells which ordinarily produce them. "Naturally-occurring" also encompasses proteins that contain or lack an NH_2-terminal methionyl group as a consequence of expression in E. coli.

"Substantially equivalent" as used throughout the specification and claims is defined to mean possessing a very high degree of amino acid residue homology (See generally M. Dayhoff, Atlas of Protein Sequence and Structure, vol. 5, p. 124 (1972), National Biochemical Research Foundation, Washington, D.C., specifically incorporated herein by reference) as well as possessing comparable biological activity.

Substantially equivalent proteins of this invention includes proteins that have a significant degree of homology with the naturally occurring protein, but have been modified to contain a limited number of mutations or deletions within the amino acid sequence. Only such modified proteins retaining the biological activity of the naturally occurring proteins

are included within the definition. Those skilled in the art are competent to prepare mutated proteins based on the known sequence of the naturally occurring proteins according to well known procedures without undue experimentation. The biological activity of such mutations may also be determined (without undue experimentation) according to the procedures described herein by those skilled in the art.

"Biological activity" as used throughout the specification and claims refers to the natural neurotrophic activity of the CNTF proteins of this invention. One measure of the biological activity of CNTF accepted by those skilled in the art is the ability to support the survival in cell cultures of chick embryonic ciliary ganglia as described in U.S. patent 5,011,914. A protein is considered to have the same or comparable biological activity as the naturally occurring proteins of this invention if the protein has a specific activity within two orders of magnitude as the naturally-occurring proteins of this invention.

Particularly preferred CNTFs of the present invention are the naturally-occurring proteins that have previously been described in a currently pending United States patent application. This application is U.S. Patent Application Serial No. 07/857,544 filed March 24, 1992 of Collins et al., which is entitled "Purified Ciliary Neurotrophic Factor." (See also, U.S. patents 5,011,914; 5,141,856; and 4,997,929). Other preferred forms of CNTF are described in U.S. Patent Application Serial No. 07/753,176 filed August 30, 1992 of Collins et al., which is entitled "Purification of Recombinant Ciliary Neurotrophic Factor and C-Terminal Truncated Ciliary Neurotrophic Factor". Each of these patents and patent applications are specifically incorporated herein by reference.

The nucleic acid sequences of the genes encoding human and animal CNTFs and the amino acid sequences of

such proteins are given in U.S. patent numbers 4,997,929 and 5,141,856. The present invention encompasses non-glycosylated forms of CNTF as well as truncated forms of the naturally-occurring and recombinant CNTF proteins as described in the above patents. In a further embodiment, CNTF is modified by attachment of one or more polyethylene glycol (PEG) or other repeating polymeric moieties. In the preferred embodiment of this invention the CNTF used is naturally-occurring recombinant human CNTF (rhCNTF).

Methods for producing the CNTFs of this invention are also disclosed in the above-mentioned patents. One disclosed method consists of isolating CNTF from various sources, such as peripheral nerve tissues. A second disclosed method involves isolating the genes responsible for coding CNTF, cloning the gene in suitable vectors and cell types, and expressing the gene in order to produce the CNTF. The latter method, which is exemplary of recombinant DNA methods in general, is a preferred method of the present invention. Recombinant DNA methods are preferred in part because they are capable of achieving comparatively higher amounts at greater purity.

Preferably, the above described CNTFs are produced by the aforementioned method in "substantially pure" form. By "substantially pure" it is meant that CNTF, in an unmodified form, has comparable biological activity to the purified CNTF described in United States patent 4,997,929. It is to be recognized, however, that derivatives of CNTF may have different specific activities. In a preferred embodiment of the present invention, a therapeutic composition comprising CNTF is administered in an effective amount to patients in order to effectively treat the symptoms of ALS.

Because it is possible that the function of the preferred CNTFs is imparted by one or more discrete and separable portions of the CNTF protein, it is also

envisioned that the method of the present invention could be practiced by administering a therapeutic composition whose active ingredient consists of that portion (or those portions) of CNTF which controls (or control) CNTF function.

The therapeutic composition of the present invention is preferably administered parenterally by injection. In the most preferred embodiment the parenteral administration is subcutaneous. Also, other effective administration forms, such as parenteral slow-release formulations, intrathecally by continuous infusion from an implanted pump, inhalant mists, orally active formulations, or suppositories, are also envisioned. The CNTF of this invention is preferably formulated with a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier is a carrier that is not harmful to the patient and does not degrade, deactivate, or in any other way hinder the effects of the CNTF. One preferred carrier is physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers may also be used. In one preferred embodiment it is envisioned that the carrier and the CNTF constitute a physiologically-compatible, slow-release formulation. The primary solvent in such a carrier may be either aqueous or non-aqueous in nature. In addition, the carrier may contain other pharmacologically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmacologically-acceptable excipients for modifying or maintaining the stability, rate of dissolution, release, or absorption of the CNTF. Such excipients are those substances usually and customarily employed to formulate dosages for parenteral administration in either unit dose or multi-dose form or for intrathecal delivery by

continuous or periodic infusion from an implanted pump or intrathecally by periodic injection.

Once the therapeutic composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder. Such formulations may be stored either in a ready to use form or requiring reconstitution immediately prior to administration. The preferred storage of such formulations is at temperatures at least as low as 4°C and preferably at -70°C. It is also preferred that such formulations containing CNTF are stored and administered at or near physiological pH. It is presently believed that storage and administration in a formulation at a pH below approximately pH 5.5 and above approximately pH 8.0 is undesirable.

Preferably, the manner of parenterally administering the formulations containing CNTF is via a subcutaneous or intramuscular route. The most preferred administration is parenterally via a subcutaneous route. To achieve the desired dose of CNTF, single or repeated subcutaneous or intramuscular injections may be administered. It is believed that the administration of CNTF in doses below approximately 0.005 μg/kg/day may not be effective, while the administration of doses of greater than 10mg/kg/day may have undesirable side effects. In a preferred embodiment of the invention the dose of CNTF is between 0.5-50 μg/kg/day. To treat or prevent the progression of ALS, it may be desirable to administer the CNTF periodically. The periodic administrations may constitute monthly, bi-weekly, weekly, daily or hourly regimes. The required frequency of administration will be apparent to those treating the patient based on standard observational techniques.

In Example 2 below, patients suffering from ALS were administered daily injections of rhCNTF in dosages

including 2 μg/kg/day, 5 μg/kg/day, 10 μg/kg/day and 20 μgkg/day. The progression of the ALS symptoms in the patients were monitored by the TQNE evaluation. Surprisingly, as seen in the results shown in Tables III and IV, the most positive trends were seen in the patient group which had been administered 2 μg/kg/day. Thus, in a preferred embodiment of the invention, patients suffering from ALS are administered rhCNTF in doses of less than about 10 μg/kg and preferable about 2-3 μg/kg. Further, in the preferred embodiment the rhCNTF is administered once daily.

It is also contemplated that certain formulations containing CNTF are to be administered orally. Preferably, CNTF which is administered in this fashion is encapsulated. The encapsulated CNTF may be formulated with or without those carriers customarily used in the compounding of solid dosage forms. Preferably, the capsule is designed so that the active portion of the formulation is released at that point in the gastro-intestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional excipients may be included to facilitate absorption of CNTF. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

Regardless of the manner of administration, the specific dose is calculated according to the approximate body weight or surface area of the patient. Further refinement of the calculations necessary to determine the appropriate dosage for treatment involving each of the above mentioned formulations is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them without undue experimentation, especially in light of the dosage information and assays disclosed herein. These dosages may be ascertained through use of the

established assays for determining dosages utilized in conjunction with appropriate dose-response data.

According to the present invention, a patient in need of a treatment for ALS is administered a therapeutically effective amount of CNTF. As described above, the dosage sufficient to deliver a "therapeutically effective amount" of CNTF can be determined by those of ordinary skill in the art without undue experimentation. A "therapeutically effective amount" may be defined as the amount of CNTF sufficient to give rise to subjective or objective improvements in the condition of the patient suffering from ALS.

It should be noted that the CNTF formulations described herein may be used for veterinary as well as human applications and that the term "patient" should not be construed in a limiting manner. In the case of veterinary applications, the dosage ranges should be the same as specified above.

It is understood that the application of teachings of the present invention to a specific problem or environment will be within the capabilities of one having ordinary skill in the art in light of the teachings contained herein. Examples of representative uses of the present invention appear in the following examples.

EXAMPLE 1:

A number of human subjects with amyotrophic lateral sclerosis (ALS) were given subcutaneous doses of recombinant human CNTF (rhCNTF) as part of an open-label, ascending study assessing the safety, tolerability and pharmacokinetics of CNTF. During a portion of this study, patients were given a single subcutaneous administration of rhCNTF each day for 28 days. (The rhCNTF was prepared in an E. coli expression system as described in U.S. patent numbers

4,997,929 and 5,141,856 and U.S. patent application serial no. 07/753,176).

Inclusion criteria: Patients included in the test met the following criteria:

1. Unequivocal diagnosis of uncomplicated ALS
2. The patient was male or female between the ages of 21-85 years.
3. The patient, if female, was postmenopausal for at least two years, surgically sterile, or, if the patient was of childbearing potential, she had been practicing a method of birth control considered effective and medically acceptable by the investigator for a minimum of 2 months prior to the study and at least 2 months after the study ended.
4. The patient was an outpatient at the time of enrollment.
5. The patient was willing to be housed for 7 days in a medical unit, and was able to comply with the study visit schedule.
6. The patient's laboratory values for white blood cells (WBCs) and differential, hemoglobin, hematocrit, platelets, serum electrolytes, SGOT, SGPT, alkaline phosphatase, BUN an creatinine, total bilirubin, and urinalysis were within clinically acceptable limits.
7. The general physical condition of the patient was such that the investigator considered the patient to be acceptable for this study and would survive at least six months.
8. The patient was given an informed consent or assent that has been approved by an institutional review board.

Exclusion criteria: Patients were excluded from the

test if they met any of the following criteria:

1. The patient has uncontrolled (over the last 30 days), clinically significant cardiovascular, pulmonary, endocrine, or gastrointestinal disease. The patient has clinically significant hematologic, metabolic, hepatic, or renal disease.
2. The patient's FVC and/or FEV_1 is $\leq$ 40% of predicted.
3. The patient has evidence for GM1 antibodies, paraproteinemia, familial ALS, or "pure" motor syndromes (Spinal muscular atrophy, etc.)
4. The patient is lactating or pregnant.
5. The patient has received another investigational drug within the past 30 days.
6. The patient has any other major neurologic disease in addition to ALS.
7. The patient has had a major surgical operation or severe infection within one month prior to the day of dosing.
8. The patient has a history of recent ethanol or drug abuse, or noncompliance with treatment on other experimental protocols.
9. The patient has limited mental capacity such that he/she cannot provide written informed consent, information regarding adverse experiences or comply with evaluation procedures.

Clinical Supplies

The rhCNTF was supplied in a sterile solution ready for injection. This solution was comprised of a Tris (10mM) and sodium phosphate buffered solution (10mM) at pH 7.2, containing 205 mM sodium chloride, 10% v/v glycerol, 0.2% w/w polysorbate 80, and 0.1% human serum albumin. The concentration of rhCNTF was

either 1.0 mg/mL or 4 mg/mL. The rhCNTF and placebo were packaged in 3 cc glass vials with Teflon-faced butyl rubber stoppers. Each vial contained 1.7 mL of formulated material. This study also used a placebo which was the vehicle for dilution of rhCNTF. The rhCNTF and placebo were stored frozen at -20 or -70 degrees Celsius. Frozen, formulated rhCNTF and placebo were thawed at room temperature. Prior to use, the liquid formulations were gently inverted several times to afford a homogenous solution.

Dosing of rhCNTF was performed with a 1 or 3 cc syringe fitted with a 27 gauge needle. In order to administer very low doses, for which the volume of 1 mg/mL solution is too small to measure accurately, a solution of 0.1 mg/mL was produced by adding 0.43 mL of 1 mg/mL solution to one vial of placebo (1.7 mL).

Anecdotal Results

Groups of six patients each were daily injected with 0.002, 0.005, 0.01 or 0.02 mg/kg of rhCNTF. 7 placebo patients at various dosage amounts were included in the study. Anecdotal results of the patients completing the 28 day regime are given below in Table I.

In summary, 2 of the 7 placebo patients experienced subjective improvement. Subjective improvement or a decrease in hyperreflexia, cramps or fasciculations on exam was reported by 15 of the 21 ALS patients receiving rhCNTF who completed the trial.

CPK Results

Serum levels of creatine phosphokinase (CPK) were measured in the test group of patients at various times for most patients, levels of serum CPK were determined prior to the initiation of administration, on the day the treatment began and weekly thereafter. The results of these measurements are shown in Table II below. In

Table II, under the column Drug/Placebo, D represents patients who received CNTF and P represents patients who received placebo, and the number represents the dosage of CNTF in mg/kg/day.

In summary, in patients who received daily injections of rhCNTF, serum CPK levels decreased in 5 of 6 patients, receiving 0.005 mg/kg/day of rhCNTF, 6 of 6 patients receiving 0.01 mg/kg/day of rhCNTF and 3 of 3 patients receiving 0.02 mg/kg/day. Patients receiving placebo showed no obvious correlations to a decrease in serum CPK levels.

It is reasonable to conclude from this data that the reduction in CPK correlates to a reduction in muscle damage or breakdown. This result may be a function of a primary protective effect on muscle cells or from a secondary protective effect from improvement in the function of innervating motor neurons. Either explanation is possible in that CNTF receptors are found on both neurons and on muscle cells.

Example 2

ALS patients meeting the inclusion and exclusion criteria set forth in Example 1 were given a daily single subcutaneous administration of rhCNTF. The condition of the patients in the study was monitored by TQNE.

The Neuromuscular Research Unit at the New England Medical Center has developed a standarzied test battery to measure the clinical deficit in ALS, the Tufts Quantitative Neuromuscular Exam (TQNE). See, Andres, PL, et al. Neurology 1986; 36: 937-941 (Quantitative motor assessment in amyotrophic lateral sclerosis); and Andres, PL, et al. Neurology 1988; 38: 409-413 (Use of composite scores (Megascores) to measure deficit in ALS). The major component of the TQNE is the measurement of maximum voluntary isometric contraction (MVIC) of 10 muscle groups in the arms and 10 muscle

groups in the legs using a strain guage tensiometer. The TQNE is most commonly used to measure disease progression and efficacy of investigational agents.

The TQNE measurements obtained at a given visit are transformed to megascores as described in Andres 1988 reference supra, by averaging the Z-transformed items (the raw score minus the mean, divided by the standard deviation of the TUFTS ALS population for that score) that compose that category.

Table 3 shows TQNE changes over 28 days of treatment in patients receiving daily subcutaneous injections of rhCNTF. The left column lists muscle groups and their respective megascores. Each column group to the right of this is a dose level (placebo, 2 5, 10, and 20 μg/kg/day). Individual columns group in each are patients who had evaluable TQNEs at baseline and at Day 28. A "+" sign demonstrate a 28-day score better than the baseline score. A "-" sign demonstrates a 28-day score worse than the baseline score. "@" signifies no changes. Table 4 shows a summary table of megascore improvement per dose group.

Although changes in this small number of patients are not statistically significant, there is a positive trend in the 2 μg/kg/day dose group.

TABLE I

DOSE LEVEL	PATIENT SUMMARY
0.002 mg\kg\day	Pt. 2-0002; walking improved (more upright, longer distances) by 2nd week of study, lasted until 3 weeks after study. Pt. 2-0004; decreased frequency of falling; before study, OOD; during study, 4 times over 28 days. One week after CNTF stopped, returned to OOD. Pt. 2-0006; improved movement of toes on left foot, still present one month after stopping CNTF. Placebo; improvement in speech and climbing stairs, as well as decreased fasciculations. Pt. 3-0001; hyperreflexia diminished. Pt. 3-0002; "perceived improvement"
0.005	Placebo; improved strength and longer ability to use walker. Pt. 4-0101; fewer cramps Pt. 4-0102; fewer fasciculations Pt. 3-0104; strength improved, walked without cane. Pt. 3-0105; cramps stopped after day 2, walking and speed improved
0.01	Pt. 2-0203; by second week, reported improved ability to use a straw (pull liquid into mouth), which persisted 2 weeks after end of study. Pt. 2-0201; fewer cramps and fasciculations, more hand motility Pt. 2-0203; fewer fasciculations Pt. 2-0204; fewer fasciculations
0.02	Pt. 5-0301; increased strength in arms and hands, started within several days after starting CNTF. Also, not dropping things as much. Neuro exam unchanged. Pt. 3-0304; improved left foot/leg strength, hyperreflexia diminished, less dependent on cane.

TABLE II

Patient	Drug/ Placebo	CPK						% Change
		Screen	0	7	14	21	28	
001	D = .002	435	---	200	337	350	678	↑+56%
002	D = .002	---	162	62	73	98	93	↑+43%
003	P = .002	858	722	572	486	482	403	↓-53%
004	D = .002	1231	1175	535	946	1146	1387	↑+13%
005	P = .002	244	250	177	209	185	174	↓-29%
006	D = .002	367	368	374	297	236	286	↓-22%
007	D = .002	616	270	217	220	257	270	No Change
008	D = .002	377	462	178	252	300	295	↓-22%
009	D = .005	---	242	107	112	142	120	↓-50%
010	P = .005	522	440	431	447	462	512	↓-2%
011	P = .005	348	384	317	434	277	368	No Change
012	D = .005	261	221	192	205	D/C	D/C	↓-21%
013	D = .005	176	---	81	111	79	D/C	↓-55%
014	D = .005	231	219	148	147	154	141	↓-39%
015	D = .005	---	291	---	165	157	129	↓-56%
016	D = .005	---	298	194	162	206	115	↓-61%
017	D = .01	76	65	74	50	37	23	↓-70%
018	P = .01	46	49	50	55	43	46	No Change
019	D = .01	---	80	53	62	38	D/C	↓-52

TABLE II CONT'D.

Patient	Drug/ Placebo	CPK						% Change
		Screen	0	7	14	21	28	
020	D = .01	559	596	228	210	275	345	↓-38%
021	D = .01	---	582	269	138	---	128	↓-78%
022	P = .01	219	234	275	---	225	---	No Change
023	D = .01	330	338	256	147	149	143	↓-57%
024	D = .01	178	147	111	56	31	32	↓-82%
025	D = .02	304	372	269	D/C	D/C	D/C	↓-12%
026	D = .02	331	378	129	151	D/C	D/C	↓-54%
027	D = .02	189	212	124	32	37	D/C	↓-80%
028	P = .02	165	238	187	180	179	161	No Change

TABLE III

SUMMARY OF TQNE CHANGES FOR DAY 28 VS. BASELINE

FOR DAILY DOSING

DOSE GROUP

	Placebo					2 µg/kg/day						5 µg/kg/day					10 µg/kg/day			20 µg/kg/day	
	1	2	3	4	5	1	2	3	4	5	6	1	2	3	4	5	1	2	3	1	2
L sh ex	-	+	-	+		+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-
R	+	+	-	-	-	+	-	+	-	+	+	-	-	+	-	+	-	-	-	+	-
L sh fl	+	+		-		-	+	+	-	-	+		+	+	-	-	+	-	-	-	-
R	+	+	+	-	-	+	+	+	+	+	+		-	-	-	-	+	-	-	+	-
L el ex	+	+	+	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
R	+	+	-	-	+	-	-	+	+	+	+	-		-	-	+	-	-	-	-	-
L el fl	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
R	-	+	-	-	-	-	+	+	+	+	+	-		-	-	+	-	-	-	-	-
L grip	-	-	-	-		-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-
R	+	+	+	+		-	-	-	+	a	+	+		+	a	-	-	-	-	+	-
Mega	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-
L dorsi	+	-	-	+	+	-	+			-	-	-	-	+	-	-	+	-	-	-	-
R	-	-	-	+	-	-	+	-		+	-	-	-	+	+	-	-	+	-	-	-
L kn ex	-	+	-	-	-	+	+	+	+	-	+	-	+	+	-	-	-	-	-	-	-
R	+	+	-	-	-	+	+	-	+	+	+	-	+	+	-	+	-	-	+	-	-

TABLE III CONT'D.

	Placebo					2 µg/kg/day						5 µg/kg/day					10 µg/kg/day			20 µg/kg/day	
L kn fl	+	-	+	+	-	-	-	+	-		-	-	+	-	-	-	-	+	-	-	-
R	+	-	+	+	+	-	-	+	-		+	+	+	+	-	-	+	-	-	-	-
L hip fl	-	+	-	-	-	+	+	-	+	+	+	-	+	+	-	-	-	-	-	-	-
R	-	+	-	-	-	+	-	+	+	+	+	-	+	+	-	+	-	-	-	-	-
Mega	+	-	-	+	-	+	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-
L peg	+	-		-		-	+	+	+	-	+	@	-	+		@	+	@	-	-	+
R	-	-		+	-	-	+	@	-	+	+	@		+		+	-	-	+	-	
Mega	+	-		+	-	-	+	+	-	+	+	@	-	+		+	-	-	-	-	-
FVC	-	-	+	-	-	-	+	+	+	-	+	+	+	+	-	-	-	-	-	+	-
Pa	@	-	+	+	+	@	@	+	@	@	@	-	@	-		+	-	+	-	@	@
Pata	@	-	+	+	-	@	@	+	+	-	@	-	@	+		+	-	+	+	@	@
Mega	@	-	+	+	-	@	@	+	+	-	@	-	@	+		+	-	+	+	@	@

+ = improvement over 28 days

- = worsening over 28 days

@ = no changes over 28 days

TABLE IV

TQNE DATA SUMMARY; BASELINE VS. DAY 28 MEGASCORES

Number of patients whose scores improved

dose level (μg/kg/day)

Mega	**Placebo**	**2 μg/kg**	**5 μg/kg**	**10 μg/kg**	**20 μg/kg**
Arm	2/5	4/6	0/5	0/3	0/2
Leg	2/5	4/6	2/5	0/3	0/2
Peg	2/5	4/6	2/5	0/3	0/2
FVC	1/5	4/6	3/5	0/3	1/2
Bulbar	2/5	2/6	2/5	2/3	0/2

CLAIMS:

1. A method for the treatment of amyotrophic lateral sclerosis (ALS) in patients suffering therefrom comprised of the administration of a therapeutically effective amount of ciliary neurotrophic factor (CNTF) in a pharmaceutically acceptable carrier.

2. The method of claim 1 wherein said CNTF is human CNTF.

3. The method of claim 1 wherein said CNTF is naturally occurring CNTF.

4. The method of claim 1 wherein said CNTF is administered parenterally.

5. The method of claim 4 wherein said CNTF is administered subcutaneously.

6. The method of claim 4 wherein said CNTF is administered intramuscularly.

7. The method of claim 1 wherein said CNTF is administered in a dose of between 0.005 μg/kg/day and 10 mg/kg/day.

8. The method of claim 7 wherein said CNTF is administered in a dose of between 0.5 and 50 μg/kg/day.

9. A method for the treatment of amyotrophic lateral sclerosis (ALS) in patients suffering therefrom comprised of the administration of a dose of less than about 10 μg/kg/day of ciliary neurotrophic factor (CNTF) in a pharmaceutically acceptable carrier.

10. The method of claim 9 wherein said CNTF is administered in a dose of about 2-3 μg/kg/day.

11. A method for the treatment of amyotrophic lateral sclerosis (ALS) in patients suffering therefrom comprised of the subcutaneous administration of a dose of about 2-3 μg/kg/day of recombinant human ciliary neurotrophic factor (rhCNTF) in a pharmaceutically acceptable carrier.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01227

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/02
US CL :514/12, 21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Medline, BIOSIS, EMBASE, APS, WPI
Search terms: ALS, CNTF, administration, treatment, neuron, nervous system

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,011,914 (COLLINS et al.) 30 April 1991, see entire document.	1-11
Y	US, A, 4,997,929 (COLLINS et al.) 05 March 1991, see entire document.	1-11
Y	US, A, 4,923,696 (APPEL et al.) 08 May 1990, see entire document.	1-11
Y	US, A, 5,093,317 (LEWIS et al.) 03 March 1992, see entire document.	1-11
Y	L.S. Goodman et al., "THE PHARMACOLOGICAL BASIS OF THERAPEUTICS", published 1975 by Macmillan Publishing Company, Inc. (N.Y.), pages 1-46, see entire document.	1-11

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
03 May 1994	18 MAY 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Jacqueline G. Krikorian Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01227

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Science, Volume 246, issued 24 November 1989, L.H. Lin et al., "Purification, cloning, and expression of ciliary neurotrophic factor (CNTF)", pages 1023-1025, see entire document.	1-11
X, P Y	Neurology, Volume 43, Number 4, Supplement 2, issued April 1993, B.R. Brooks et al., "Recombinant human ciliary neurotrophic factor (rHCNTF) in amytrophic lateral sclerosis (ALS) patients: Phase I-II safety, tolerability and pharmacokinetic studies, page A416, abstract no. 9905see entire abstract.	1,2,4,5 3,6-11
X Y	WO, A, 91/04316 (MASIAKOWSKI et al.) 04 April 1991, see entire document, especially claim 109.	1-4 5-11