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(54) Title: METHODS FOR PREVENTING, TREATING OR DELAYING CARDIAC TOXICITY USING NEUREGULIN

(57) Abstract: The present invention provides methods for preventing, treating or delaying cardiac toxicity in a mammal to which such prevention, treatment or delay is needed or desirable, comprising administering to a mammal *in vivo* an effective amount of a prophylactic or a therapeutic agent and an effective amount of: (i) a neuregulin protein or a functional fragment thereof; (ii) a nucleic acid encoding a neuregulin protein or a functional fragment thereof; or (iii) an agent that enhances production or function of said neuregulin, whereby the cardiac toxicity associated with administration of said prophylactic or therapeutic agent is prevented, treated or delayed.

METHODS FOR PREVENTING, TREATING OR DELAYING CARDIAC TOXICITY USING NEUREGULIN

Technical Field

The present invention relates to methods for preventing, treating or delaying cardiac toxicity in mammals, particularly in humans. More particularly, the present invention provides neuregulin-based *in vivo* methods for preventing, treating or delaying cardiac toxicity associated with a prophylactic or therapeutic agent.

Background Art

Doxorubicin or adriamycin (ADM) belongs to anthracycline anticancer agents with a broad spectrum and potent anti-tumor activity. It has been widely used in clinical practice to treat various malignant tumors such as lung cancer, breast cancer, bladder carcinoma, testis carcinoma, thyroid cancer, soft tissue carcinoma, osteosarcoma, neuroblastoma, acute leukemia, malignant lymphoma, gastric carcinoma, liver cancer, esophageal carcinoma, and cervical carcinoma. ADM acts through incorporation with DNA to inhibit DNA synthesis. The inhibition effect is observed in S, M, G1 and G2 phases, but the activity is most active in S phase. It has been discovered that a higher dose of chemotherapy agents produces better clinical efficacy and longer survival in unit time for breast cancer patients, whether metastatic or post-operative chemotherapy. Other studies have confirmed the importance of higher dose and dose potency, as well as the relation between dose, dose potency and efficacy.

However, the application of ADM is restricted by its toxic side effects. The toxicity of ADM includes bone marrow depression. In about 60-80% patients, leucocyte and platelet counts decrease to the lowest level 10-15 days after administration, and recover to normal levels 21 days later. Major digestive tract reaction are nausea, vomiting, anorexia, gastritis and even ulcer and stomatitis. Alopecia also occurs in nearly all patients treated with ADM, although this effect can be reversed after withdrawal.

More importantly, the major factor preventing clinical application of higher dose ADM and other anthracycline agents is cardiac toxicity. Cardiac toxicity of ADM may result from the production of oxygen-derived free radicals. The semiquinone group in ADM can induce an

oxidoreduction reaction, which leads to enhanced lipid peroxidation, contributing to cellular damage. The free radicals damage cell membrane and organelle membrane, and modify the function of membrane proteins and enzyme activity. These changes can lead to intracellular calcium overload, inhibition of DNA and protein synthesis, and energy metabolism disorder, which inevitably harm the cardiac contraction and relaxation process.

Cardiac toxicity occurs when ADM accumulates in the body because ADM has a higher affinity for cardiac tissue than for other tissues. Thus, the heart is more vulnerable to the toxic effect of ADM. Cardiac toxicity may be acute or chronic. Clinical features of acute cardiac toxicity include cardiac functional changes, such as sinus tachycardia, arrhythmia, conduction block, ST-T segment alteration, etc. Various arrhythmia may be present at early stages of ADM therapy. Acute cardiac toxicity can also include decrease of the left ventricular ejection fraction (LVEF), as well as decreases in stroke volume (SV), cardiac output (CO) and cardiac index (CI). ADM has also been suggested to inhibit the contraction and pumping ability of the left ventricle. Chronic cardiac toxicity include irreversible congestive heart failure. Once a person suffers from congestive heart failure, the mortality decreases to about 30 % to 50 %. Because of the cardiac toxicity associated with ADM, some patients have to terminate ADM therapy, or reduce the dosage or length of ADM use, affecting the efficacy of ADM therapy.

Studies have been conducted to find ways to reduce the toxicity of ADM, while maintaining the therapeutic effect. Certain measures, such as decreasing the total dose of ADM or administering myocardial nutrients such as a high dose of vitamin C, may be helpful in protecting myocardium. Regular blood transfusion and administering an iron chelating agent ICRF-187 also have some effect in protecting myocardium. However, although these supportive agents are beneficial to the cardiac muscle, they have little effect on the cardiac toxicity induced by ADM. Thus, there exists a need in the art for preventing, treating or delaying the cardiac toxicity of ADM without affecting its efficacy.

Disclosure of the Invention

It is an object of the present invention to provide methods for preventing, treating or delaying cardiac toxicity associated with prophylactic or therapeutic agents. In particular, it is an

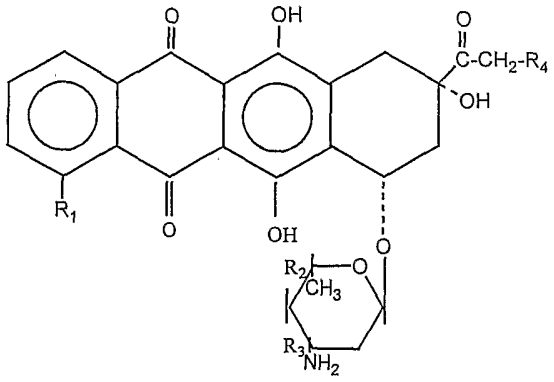
object of the present invention to provide methods for preventing, treating or decreasing cardiac toxicity of drug-induced cardiomyopathy.

The present invention provides methods for preventing, treating or delaying cardiac toxicity in a mammal to which such prevention, treatment or delay is needed or desirable, comprising administering to a mammal *in vivo* an effective amount of a prophylactic or a therapeutic agent and an effective amount of: (i) a neuregulin protein or a functional fragment thereof; (ii) a nucleic acid encoding a neuregulin protein or a functional fragment thereof; or (iii) an agent that enhances production or function of said neuregulin, whereby the cardiac toxicity associated with administration of said prophylactic or therapeutic agent is prevented, treated or delayed.

The present invention can be used to prevent, treat or delay any clinical manifestations of cardiac toxicity known to one of ordinary skill in the art, including but not limited to acute or chronic cardiac toxicity. For example, the present invention can be used to prevent, treat or delay tachycardia, arrhythmia and congestive heart failure. In a particular embodiment, the present invention can be used to prevent, treat or delay clinical manifestations of acute cardiac toxicity such as sinus tachycardia, arrhythmia, conduction block and ST-T segment alteration, as well as decreases in the left ventricular ejection fraction (LVEF), stroke volume (SV), cardiac output (CO) or cardiac index (CI). The present invention can also be used to prevent, treat or delay cardiac toxicity comprising the inhibition of the contracting and pumping ability of the left ventricle.

The present methods can be used to prevent, treat or delay cardiac toxicity associated with any prophylactic or therapeutic agent. In one embodiment, the prophylactic or therapeutic agent produces oxygen-derived free radicals, which cause cardiac toxicity. In another embodiment, the prophylactic or therapeutic agent enhances lipid peroxidation, which causes cardiac toxicity.

The present methods can be used to prevent, reduce or delay cardiac toxicity associated with anti-neoplasm agents. The anti-neoplasm agent is preferably an anthracycline. In a specific embodiment, the anti-neoplasm agent has the following formula I:



wherein R1 is methoxy or hydrogen; and R2, R3 and R4 are hydroxy or hydrogen.

Non-limiting examples of anthracycline for use in the present methods include adriamycin (or doxorubicin), daunorubicin, epirubicin, idarubicin, mitoxantrone, mitomycin, bleomycin, cyclophosphamide, fluorouracil, actinomycin D and vincristine. In one aspect, the present invention is directed to the use of neuregulin as an anti-cardiotoxic agent for preventing, treating or delaying cardiac toxicity induced by chemotherapeutic agents such as adriamcyin (ADM), alone or in combination with another reagent.

The present methods can be used to prevent, reduce or delay cardiac toxicity associated with antipsychotic agents. The antipsychotic agent can be chlorpromazine, perphenazine or trifluperazine.

The present methods can be used to prevent, reduce or delay cardiac toxicity associated with tricyclic antidepressants. The tricyclic antidepressant can be chlorimipramine, amitriptyline or doxepin.

The present methods can be used to prevent, reduce or delay cardiac toxicity associated with interferon, *e.g.*, interferon-α, or interleukin, *e.g.*, interleukin-2.

The present methods can be used to prevent, reduce or delay cardiac toxicity associated with anti-infectious agents, *e.g.*, emetine.

The neuregulin agent for use in the present methods can be neuregulin 1, neuregulin 2, neuregulin 3, or neuregulin 4. In a particular embodiment, the neuregulin for use in the present methods is neuregulin α2 or neuregulin β2. In another embodiment, the neuregulin fragment is a neuregulin β2 comprising an amino acid sequence set forth in SEQ ID NO:2.

The neuregulin agent can be administered as a protein or a functional fragment thereof. The neuregulin agent can also be administered as a nucleic acid encoding a neuregulin protein or a functional fragment thereof. Any agent that enhances production or function of neuregulin can also be administered. The neuregulin agent can be administered alone or with a pharmaceutically acceptable carrier or excipient. The neuregulin protein or a functional fragment thereof, or a nucleic acid encoding a neuregulin protein or a functional fragment thereof, or an agent that enhances production or function of said neuregulin, can be administered prior to, concurrently with, or subsequent to the administration of the prophylactic or therapeutic agent.

In one embodiment, the prophylactic or therapeutic agent is administered in an amount that is higher than a maximally allowed amount when the prophylactic or therapeutic agent is administered in the absence of the neuregulin protein or a functional fragment thereof, or a nucleic acid encoding a neuregulin protein or a functional fragment thereof, or an agent that enhances production or function of said neuregulin.

In a particular embodiment, the present invention provides methods for preventing, treating or delaying cardiac toxicity in a human to which such prevention, treatment or delay is needed or desirable. Preferably, the human has a malignant tumor such lung cancer, breast cancer, bladder carcinoma, testis carcinoma, thyroid cancer, soft tissue carcinoma, osteosarcoma, neuroblastoma, acute leukemia, malignant lymphoma, gastric carcinoma, liver cancer, esophageal carcinoma, or cervical carcinoma.

Brief Description of the Drawings

Figure 1 illustrates the construction of an engineered bacterial strain for recombinantly producing a neuregulin protein.

Figure 2 shows the results of PCR amplification of a human neuregulin gene. Lane 1 depicts a 183bp neuregulin gene obtained by RT-PCR; lanes 2 and 3 are DNA markers.

Figure 3 shows a physical map of plasmid PET22b.

Figure 4 depicts the identification of recombinant plasmid by endonuclease digestion. Lanes 1 and 3 are DNA markers; lane 2 depicts fragments after enzyme digestion.

Figure 5 depicts screening for expression of an engineered strain after induction by isopropyl thiogalactoside (IPTG). Lane 1 depicts a protein marker; lane 2 depicts an engineered

strain without induction; lanes 3-6 depict an engineered strain after one, two, three and four hours induction, respectively; and lanes 7-9 depict different strains with induction.

Figure 6 shows the therapeutic effect of neuregulin on the survival of Sprague-Dawley derived (SD) rats. In this figure, the symbols represent the results for different groups: (♦)for a normal group; (▲) for a negative control group; (x) for a treatment group receiving 233 μg/kg neuregulin; (*) for a treatment group receiving 23.3 μg/kg neuregulin; (•) for a treatment group receiving 2.33 μg/kg neuregulin; and (+) for a treatment group receiving 0.233 μg/kg neuregulin.

Figure 7 shows a statistical comparison (dp/dt) amongst a normal group (1); positive and negative control groups (2 and 3, respectively); and different treatment groups receiving 233 μg/kg neuregulin, 23.3 μg/kg neuregulin, 2.33 μg/kg neuregulin, and 0.233 μg/kg neuregulin (4-7, respectively).

Figure 8 compares the ratio of heart weight and body weight amongst a normal group (1); positive and negative control groups (2 and 3, respectively); and different treatment groups receiving 233 μg/kg neuregulin, 23.3 μg/kg neuregulin, 2.33 μg/kg neuregulin, and 0.233 μg/kg neuregulin (4-7, respectively).

Figure 9 compares the thickness of left ventricular wall amongst a normal group (1); positive and negative control groups (2 and 3, respectively); and different treatment groups receiving 233 μg/kg neuregulin, 23.3 μg/kg neuregulin, 2.33 μg/kg neuregulin, and 0.233 μg/kg neuregulin (4-7, respectively).

Modes of Carrying Out the Invention

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the subsections that follow.

A. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. All patents, applications, published applications and other publications referred to herein are incorporated by reference in their entirety. If a definition set forth in this section is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published

applications and other publications that are herein incorporated by reference, the definition set forth in this section prevails over the definition that is incorporated herein by reference.

As used herein, "a" or "an" means "at least one" or "one or more."

As used herein, the terms "neuregulin" or "neuregulin agent" refer to proteins or peptides that can activate erbB2/erbB4 or erbB2/erbB3 heterodimer protein kinases. Neuregulin products include, but are not limited to, neuregulin isomers, neuregulin EGF domains, neuregulin mutant and any neuregulin-like products that can activate the above-mentioned receptors. Non-limiting examples of neuregulin or neuregulin agents include a neuregulin protein or a functional fragment thereof; a nucleic acid encoding a neuregulin protein or a functional fragment thereof; and an agent that enhances production or function of said neuregulin.

As used herein, the term "neuregulin protein" encompasses a neuregulin protein and a neuregulin peptide.

As used herein, the term "neuregulin nucleic acid" encompasses neuregulin nucleic acid and neuregulin oligonucleotide.

As used herein, "epidermal growth factor-like domain" or "EGF-like domain" refers to a polypeptide motif encoded by the neuregulin gene that binds to and activates erbB2, erbB3, erbB4, or combinations thereof. In particular, the EGF-like domain bears a structural similarity to the EGF receptor-binding domain as disclosed in WO 00/64400, Holmes et al., *Science*, 256:1205-1210 (1992); U.S. Patent Nos. 5,530,109 and 5,716,930; Hijazi et al., *Int. J. Oncol.*, 13:1061-1067 (1998); Chang et al., *Nature*, 387:509-512 (1997); Carraway et al., *Nature*, 387:512-516 (1997); Higashiyama et al., *J. Biochem.*, 122:675-680 (1997); and WO 97/09425.

As used herein, a "functional derivative or fragment" of neuregulin refers to a derivative or fragment of the neuregulin protein or its encoding nucleic acid that still substantially retains its anti-cardiotoxic activity. Normally, the derivative or fragment retains at least 50% of its anti-cardiotoxic activity. Preferably, the derivative or fragment retains at least 60%, 70%, 80%, 90%, 95%, 99% and 100% of its anti-cardiotoxic activity. In another embodiment, the derivative or fragment retains a higher anti-cardiotoxic activity compared to the intact neuregulin.

As used herein, an "agent that enhances production of neuregulin" refers to a substance that increases transcription and/or translation of a neuregulin gene. Alternatively, it refers to a

substance that increases post-translational modification and/or cellular trafficking of a neuregulin precursor, or a substance that prolongs the half-life of a neuregulin protein.

As used herein, an "agent that enhances function of neuregulin" refers to a substance that increases the potency of neuregulin's anti-cardiotoxic activity, or a substance that increases the sensitivity of a neuregulin's natural ligand in an anti-cardiotoxic signaling pathway. Alternatively, it refers to a substance that decreases the potency of a neuregulin's antagonist. Such an agent is not a neuregulin protein or its encoding nucleic acid.

As used herein, a "combination" refers to any association between two or among more items.

As used herein, a "composition" refers to any mixture of two or more products or compounds. It may be a solution, a suspension, liquid, powder, a paste, aqueous, non-aqueous or any combination thereof.

As used herein, "*erb*" refers to two oncogenes, *erb* A and *erb* B, associated with erythroblastosis virus (an acute transforming retrovirus).

As used herein, "heart failure" refers to an abnormality of cardiac function where the heart does not pump blood at the rate needed for the requirements of metabolizing tissues. Heart failure includes a wide range of diseases such as congestive heart failure, myocardial infarction, tachyarrythmia, familial hypertrophic cardiomyopathy, ischaemic heart disease, idiopathic dilated cardiomyopathy, and myocarditis. The heart failure can be caused by any number of factors, including ischaemic, congenital, rheumatic, or idiopathic factors. Chronic cardiac hypertrophy is a significantly diseased state which is a precursor to congestive heart failure and cardiac arrest.

As used herein, "production by recombinant means" refers to production of recombinant nucleic acid using well known methods of molecular biology, and expressing proteins encoded by cloned nucleic acids.

As used herein, the terms "vector" or "plasmid" refer to discrete elements that are used to introduce heterologous DNA into cells for either expression or replication thereof. Selection and use of such vehicles are well known within the skill of the artisan. An expression vector includes vectors capable of expressing DNAs that are operatively linked with regulatory sequences, such as promoter regions, that are capable of effecting expression of such DNA fragments. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage,

recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the cloned DNA. Appropriate expression vectors that are well known to those of skill in the art include those that are replicable in eukaryotic and/or prokaryotic cells, those that remain episomal, or those that integrate into the host cell genome.

As used herein, "a promoter region or promoter element" refers to a segment of DNA or RNA that controls transcription of the DNA or RNA to which it is operatively linked. The promoter region includes specific sequences that are sufficient for RNA polymerase recognition, binding and transcription initiation. This portion of the promoter region is referred to as the promoter. In addition, the promoter region includes sequences that modulate this recognition, binding and transcription initiation activity of RNA polymerase. These sequences may be *cis* acting or may be responsive to *trans* acting factors. Promoters, depending upon the nature of the regulation, may be constitutive or regulated. Exemplary promoters contemplated for use in prokaryotes include the bacteriophage T7 and T3 promoters, and the like.

As used herein, "operatively linked or operationally associated" refers to the functional relationship of DNA with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA. In order to optimize expression and/or *in vitro* transcription, it may be necessary to remove, add or alter 5' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation (*i.e.*, start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, *e.g.*, Kozak, *J. Biol. Chem.*, 266:19867-19870 (1991)) can be inserted immediately 5' of the start codon and may enhance expression. The desirability of, or need for, such modification may be empirically determined.

As used herein, the term "a therapeutic agent" refers to any conventional drug or drug therapies which are known to those skilled in the art, including, but not limited to prophylactic or chemotherapeutic agents.

As used herein, the term "therapeutically effective amount" refers to that amount that is sufficient to ameliorate, or in some manner reduce the symptoms associated with a disease. Such amount may be administered as a single dosage or according to a regimen. Repeated administration may be required to achieve the desired amelioration of symptoms.

As used herein, the terms "administration" or "administering" a compound refers to any suitable method of providing a compound to a subject.

As used herein, the terms "treatment" or "treating" refer to any manner in which the symptoms of a condition, disorder or disease are ameliorated or otherwise beneficially altered. Treatment also encompasses any pharmaceutical use of the compositions herein. Amelioration of symptoms of a particular disorder refers to any lessening of symptoms, whether permanent or temporary, that can be attributed to or associated with administration of the composition.

As used herein, neoplasm (neoplasia) refers to abnormal new growth or tumor growth, which may be benign or malignant. Unlike hyperplasia, neoplastic proliferation persists even in the absence of the original stimulus.

As used herein, an anti-neoplasm agent refers to any agents used to prevent the occurrence or lessen the severity of neoplasm, tumor or cancer. These include, but are not limited to, anti-angiogenic agents, alkylating agents, antimetabolites, natural products, platinum coordination complexes, anthracenedione, substituted urea, methylhydrazine derivatives, adrenocortical suppressants, hormones, antagonists, oncogene inhibitors, tumor suppressor genes or proteins, anti-oncogene antibodies, or anti-oncogene antisense oligonucleotides.

As used herein, the term "anti-psychotic agent" refers to any agents used in the treatment of psychiatric disorders. These include, but are not limited to, tricyclic phenothiazines, thioxanthenes, and dibenzepines, as well as butyrophenones and congeners, other heterocyclics, and experimental benzamides.

As used herein, the term "tricyclic antidepressants" refers to any agents used in the treatment of depression. These include, but are not limited to, agents that inhibit norepinephrine and serotonin uptake into nerve endings, and thus leading to substained facilitation of noradrenergic function in the brain.

As used herein, term "anti-infectious agent" refers to any agents used in the treatment of infectious diseases. These include, but are not limited to, agents for use against parasitic infections, bacterial and microbial infections.

B. Methods for Preventing, Treating or Delaying Cardiac Toxicity

Neuregulin has been found to enhance the differentiation of cardiac myocytes, and strengthen the combination of sarcomere and cytoskeleton, as well as intercellular cohesion (WO 00/37095). Neuregulin can also be used to detect, diagnose and treat heart diseases. In the methods of the present invention, neuregulin is used as a cardiocyte protective agent for preventing, treating or delaying cardiac toxicity due to other prophylactic or therapeutic agents.

In one aspect, the present invention is directed to a method for preventing, treating or delaying cardiac toxicity in a mammal to which such prevention, treatment or delay is needed or desirable, comprising administering to a mammal *in vivo* an effective amount of a prophylactic or a therapeutic agent and an effective amount of: (i) a neuregulin protein or a functional fragment thereof; (ii) a nucleic acid encoding a neuregulin protein or a functional fragment thereof; or (iii) an agent that enhances production or function of said neuregulin, whereby the cardiac toxicity associated with administration of said prophylactic or therapeutic agent is prevented, treated or delayed. The present method can be used for preventing, treating or delaying cardiac toxicity in any mammals, such as mice, rats, rabbits, cats, dogs, pigs, cows, ox, sheep, goats, horses, monkeys and other non-human primates. Preferably, the present method is used for preventing, treating or delaying cardiac toxicity in humans.

1. Neuregulin Agents

Any suitable neuregulin protein or a functional fragment thereof, or a nucleic acid encoding a neuregulin protein or a functional fragment thereof, can be used in the present methods. In one embodiment, the methods of the present invention uses a polypeptide fragment of a human neuregulin β 2 isomer, which contains the receptor-binding domain (*i.e.*, an EGF-class region). This polypeptide can activate the erbB receptor of the EGF receptor family and modulate its biological reactions (*e.g.*, stimulate breast cancer cell differentiation and milk protein secretion; induce the differentiation of neural crest cell into Schwann cell; stimulate acetylcholine synthesis in skeletal muscle cell; and/or improve cardiocyte survival and DNA synthesis). Neuregulin

nucleic acids and proteins can be produced by any suitable methods known in the art, including but not limited to recombinant production, chemical synthesis or a combination of both. Preferably, neuregulin nucleic acids and proteins are produced by recombinant production. (*See*, Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons, Inc. 2002).

Neuregulin variants with conservative amino acid substitutions that do not substantially alter their anti-cardiotoxic activity can also be used in the present methods. Suitable conservative substitutions of amino acids are known to those of skill in this art, and may be made generally without altering the biological activity of the resulting molecule. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity (*see e.g.*, Watson *et al. Molecular Biology of the Gene*, 4th Edition, page 224, The Benjamin/Cummings Pub. Co., 1987).

The nucleic acid encoding a neuregulin protein or a functional fragment thereof, can be used in the form of naked DNA, complexed DNA, cDNA, plasmid DNA, RNA or other mixtures thereof as components of the gene delivery system. In another embodiment, the nucleic acid encoding a neuregulin protein or a functional fragment thereof, is included in a viral vector. Any viral vectors that are suitable for gene therapy can be used. Non-limiting examples include adenovirus vectors (U.S. Patent No. 5,869,305), simian virus vectors (U.S. Patent No. 5,962,274), conditionally replicating human immunodeficiency viral vectors (U.S. Patent No. 5,888,767), retroviruses, SV40, herpes simplex viral amplicon vectors, and vaccinia virus vectors. In addition, the genes can be delivered in a non-viral vector system such as a liposome wherein the lipid protects the DNA or other biomaterials from oxidation during the coagulation.

In a specific embodiment, the neuregulin used in the present methods carries out its anti-cardiotoxic activity via binding with any of the erbB2-erbB4 receptors. In another specific embodiment, neuregulin 1, neuregulin 2, neuregulin 3 or neuregulin 4 is used in the present methods. Synonyms of neuregulin 1 include heregulin, GGF2 and p185erbB2 ligand. (*See e.g.*, WO 00/64400 and U.S. Patent Nos. 5,530,109 and 5,716,930). Both neuregulin α2 and neuregulin β2 can be used in the present methods. Preferably, a neuregulin β2 fragment comprising an amino acid sequence set forth in SEQ ID. NO:2 is used in the present methods.

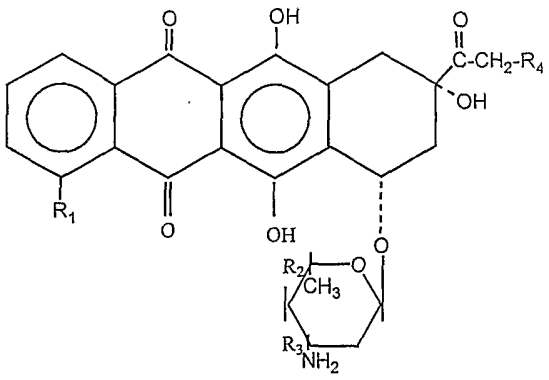
In still another specific embodiments, neuregulins or functional fragments thereof disclosed in the following patents, patent applications and GenBank databases can be used in the present

methods: U.S. Patent Nos. 6,252,051 and 6,121,415 (NRG3); 6,087,323 (neuregulin with $p185^{erbB2}$, $p185^{erbB3}$ or $p185^{erbB4}$ binding activity); 6,033,906 (neuregulin as a ligand for a receptor selected from the group consisting of $p185^{erbB2}$ and $p180^{erbB4}$); US2002002276 (chimeric ErbB heteromultimer adhesins as competitive antagonists or agonists of a neuregulin); WO01/81540 (NRG-4); WO01/64877 (NRG1); WO01/64876 (NRG1AG1); WO01/58948 (neuregulin- beta); WO01/26607 (SMDF and GGF neuregulin splice variant isoforms); WO00/70322 (CRD-neuregulin); WO00/64400; WO99/18976; WO98/02540 (chimeric ErbB heteromultimer adhesins as competitive antagonists or agonists of a neuregulin); WO96/30403; WO96/15812; BC017568 (Homo sapiens, Similar to neuregulin 4); BC007675 (Homo sapiens, neuregulin 1); AF142632 (Xenopus laevis cysteine-rich domain neuregulin-1); AF194439 (Rattus norvegicus SMDF neuregulin alpha 2a (Nrg1)); AF194438 (Rattus norvegicus SMDF neuregulin beta 1a (Nrg1)); HS2NRG12 (Homo sapiens alternatively spliced neuregulin 2 (NRG2)); HS2NRG08 (Homo sapiens alternatively spliced neuregulin 2 (NRG2)); HS2NRG07 (Homo sapiens alternatively spliced neuregulin 2 (NRG2)); AF083067 (Mus musculus neuregulin-4 short isoform (Nrg4)); AF076618 (Xenopus laevis neuregulin alpha-1); AF045656 (Gallus gallus neuregulin beta-2b); AF045655 (Gallus gallus neuregulin beta-2a); AF045654 (Gallus gallus neuregulin beta-1a); MAU96612 (Mesocricetus auratus neuregulin); AF010130 (Mus musculus neuregulin-3 (NRG3)). Preferably, neuregulin(s) disclosed in the GenBank Accession No. NT_007995 (gi:18570363) is used in the present methods.

2. Prophylactic or Therapeutic Agents

The present invention provides methods for preventing, treating or delaying cardiac toxicity associated with the administration of a prophylactic or therapeutic agent. The neuregulin agent can be administered prior to, concurrently with, or subsequent to the administration of the prophylactic or therapeutic agent. The present methods are not limited to preventing, treating or delaying cardiac toxicity associated with specific prophylactic or therapeutic agents. Non-limiting examples of prophylactic or therapeutic agents for use in the present methods include anti-neoplasm agents, antipsychotic agents, tricyclic depressants, interferons, interleukins, and anti-infectious agents.

Any anti-neoplasm agent can be used in the present methods. Preferably, the anti-neoplasm agent is an anthracyline anti-neoplasm agent having the formula:



wherein R1 is methoxy or hydrogen; and R2, R3 and R4 are hydroxy or hydrogen.

Non-limiting examples of anthracycline anti-neoplasm agent include adriamycin (or doxorubicin), daunorubicin, epirubicin, idarubicin, mitoxantrone, mitomycin, bleomycin, cyclophosphamide, fluorouracil, actinomycin D, vincristine, and derivatives thereof. (*See* Goodman & Gilman's, The Pharmacological Basis of Therapeutics, Ninth Edition, pp. 1264-1269, McGraw-Hill 1996). Other examples of anti-neoplasm agents that can be used in the present methods are described in U.S. Patent Application No. 2002/044919. Other anti-neoplasm agents include, but are not limited to, cytidine, arabinosyladenine (araC), daunomycin, methotrexate (MTX), fluorinated pyrimidines such as 5-fluorouracil (5-FU), hydroxyurea, 6-mercaptopurine, plant alkaloids such as vincristine (VCR), VP-16 and vinblastine (VLB), alkylating agent, cisplatin, nitrogen Mustard, trisamine, procarbazine, bleomycin, mitomycin C, actinomycin D, or an enzyme such as L-Asparaginase. (See Goodman & Gilman's, The Pharmacological Basis of Therapeutics, Ninth Edition, pp. 1227-1229).

Any antipsychotic agent can be used in the present methods. Non-limiting examples of antipsychotic agents include chlorpromazine, perphenazine and trifluperazine. Other examples of antipsychotic agents can be found in Goodman & Gilman's, The Pharmacological Basis of Therapeutics, Ninth Edition, pp. 400-420.

Any tricyclic antidepressant can be used in the present methods. Non-limiting examples of tricyclic antidepressants include chlorimipramine, amitriptyline and doxepin. Other examples of antipsychotic agents can be found in Goodman & Gilman's, The Pharmacological Basis of Therapeutics, Ninth Edition, pp. 431-434.

Any interferon can be used in the present methods. Preferably, the interferon is interferon-α. In one embodiment, the interferon is human interferon-α. Methods for producing interferon-α can be found in U.S. Patent Nos. 6,005,075; 5,834,235; 5,503,828; and 4,820,638.

Any interleukin can be used in the present methods. Preferably, the interleukin is interleukin-2. In one embodiment, the interleukin is human interleukin-2. Methods for producing interleukin-2 can be found in U.S. Patent No. 5,834,441; 5,795,777; 5,419,899; and 5,399,699.

Any anti-infectious agent can be used in the present methods. Preferably, the anti-infectious agent is emetine. Other examples of anti-infectious agent can be found in Goodman & Gilman's, The Pharmacological Basis of Therapeutics, Ninth Edition, pp. 965-1008.

Neuregulin agents can be administered *in vivo* (*i.e.*, administered directly into a mammal). Alternatively, the neuregulin protein or a functional fragment thereof, or a nucleic acid encoding a neuregulin protein or a functional fragment thereof, can be administered *ex vivo* (*i.e.*, administered into cells, tissues or organs, wherein such cells, tissues or organs carrying the neuregulin protein, or a functional fragment thereof, or a nucleic acid encoding a neuregulin protein, or a functional fragment thereof, can be transferred into a mammal).

C. The Formulation, Dosage and Route of Administration of Neuregulin

The formulation, dosage and route of administration of a neuregulin protein or a functional fragment thereof, or a nucleic acid encoding a neuregulin protein or a functional fragment thereof, or an agent that enhances production or function of neuregulin, preferably in the form of pharmaceutical compositions, can be determined according to the methods known in the art (*see e.g.*, *Remington: The Science and Practice of Pharmacy*, Alfonso R. Gennaro (Editor) Mack Publishing Company, April 1997; *Therapeutic Peptides and Proteins: Formulation, Processing, and Delivery Systems*, Banga, 1999; and *Pharmaceutical Formulation Development of Peptides and Proteins*, Hovgaard and Frkjr (Ed.), Taylor & Francis, Inc., 2000; *Medical Applications of Liposomes*, Lasic and Papahadjopoulos (Ed.), Elsevier Science, 1998; *Textbook of Gene Therapy*, Jain, Hogrefe & Huber Publishers, 1998; *Adenoviruses: Basic Biology to Gene Therapy*, Vol. 15, Seth, Landes Bioscience, 1999; *Biopharmaceutical Drug Design and Development*, Wu-Pong and Rojanasakul (Ed.), Humana Press, 1999; *Therapeutic Angiogenesis: From Basic Science to the Clinic*, Vol. 28, Dole et al. (Ed.), Springer-Verlag New York, 1999). Pharmaceutically acceptable compositions and methods for their administration that may be employed for use in this

invention include, but are not limited to those described in U.S. Patent Nos. 5,736,154; 6,197,801 B1; 5,741,511; 5,886,039; 5,941,868; 6,258,374 B1; and 5,686,102.

The neuregulin protein or a functional fragment thereof, or a nucleic acid encoding a neuregulin protein or a functional fragment thereof, or an agent that enhances production or function of neuregulin can be administered alone. Alternatively, the neuregulin agent can be administered with a pharmaceutically acceptable carrier or excipient. Any suitable pharmaceutically acceptable carrier or excipient can be used in the present method (*see e.g.*, Remington, The Science and Practice of Pharmacy). Non-limiting examples of pharmaceutical carrier or excipient include beta-cyclodextrin and 2-hydroxy-propyl-beta-cyclodextrin.

According to the present invention, the neuregulin protein or a functional fragment thereof, or a nucleic acid encoding the neuregulin protein or a functional fragment thereof, or an agent that enhances production or function of neuregulin, alone or in combination with other agents, carriers or excipients, may be formulated for any suitable administration route. Non-limiting examples of administering a neuregulin agent include intracavernous injection, subcutaneous injection, intravenous injection, intramuscular injection, intradermal injection, oral or topical administration. Dosage forms include tablets, troches, cachet, dispersions, suspensions, solutions, capsules, patches, and the like. (*see e.g.*, Remington, The Science and Practice of Pharmacy).

For example, the method may employ formulations for injectable administration in unit dosage form, in ampoules or in multidose containers with an added preservative. The formulations may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, sterile pyrogen-free water or other solvents, before use. Topical administration in the present invention may employ the use of a foam, gel, cream, ointment, transdermal patch, or paste.

In a specific embodiment, the neuregulin agent can be formulated for oral, rectal, topical, inhalational, buccal (*e.g.*, sublingual), parenteral (*e.g.*, subcutaneous, intramuscular, intradermal, or intravenous), transdermal administration or any other suitable route of administration. In preparing compositions for parenteral dosage form, pharmaceutical media such as water, glycols, oils, buffers, sugar, preservatives, liposomes, and the like may be used. Examples of such parenteral compositions include, but are not limited to dextrose 5% w/v, normal saline or other solutions.

The most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular neuregulin agent used.

The magnitude of a therapeutic dose in the treatment or prevention will vary with the severity of the condition to be treated and the route of administration. The dose, and perhaps dose frequency, will also vary according to age, body weight, condition and response of the individual patient. The attending physician would know how to and when to terminate, interrupt or adjust therapy to lower dosage due to toxicity, or adverse effects. Conversely, the physician would also know how to and when to adjust treatment to higher levels if the clinical response is not adequate (precluding toxic side effects).

The neuregulin protein or a functional fragment thereof, or a nucleic acid encoding a neuregulin protein or a functional fragment thereof, or an agent that enhances production or function of neuregulin, can be used in any suitable dosage range. For example, the neuregulin protein or a functional fragment thereof, or a nucleic acid encoding a neuregulin protein or a functional fragment thereof, or an agent that enhances production or function of neuregulin, can have a dosage range from about 25 μg to about 2,500 μg. The total dose of the neuregulin agent can also be administered in a vial of intravenous fluid, ranging from about 1 ml to 2000 ml. The volume of dilution fluid will vary according to the total dose administered.

Example 1

Construction of a Human Neuregulin Gene

Human neuregulin gene is located in chromosome 8P12 with about 13 exons. Recombinant neuregulin is composed of 61 amino acid, with a theoretical molecular weight of 7055D and an apparent molecular weight in SDS-PAGE electrophoresis of 6500-7000D. Recombinant neuregulin has an isoelectric point of about 6.5. Neuregulin has no glucosylated locus, and contains three disulfide bonds. The gene encoding neuregulin is suitable for expression in *E. coli*.

PET22b was selected as an expression plasmid. Human neuregulin gene was introduced into the plasmid and then *E. coli* BL21 was transformed by this plasmid. High level expression recombinant was screened out as an engineering strain for producing recombinant human neuregulin.

Total RNA and mRNA were extracted from the brain tissue of a five-month human fetus, and reversely transcribed to cDNA. RT-PCR was performed with the transcribed cDNA as the template, using a pair of primers P1 and P2 to amplify target gene. The PCR product was examined in electrophoresis on 1.5% agarose, which showed a specific 183bp DNA fragment (Figure 2).

Example 2

Identification of Recombinant Plasmid by Endonuclease Digestion

Calcium chloride sedimentation method was applied to clone human neuregulin gene into expression plasmid PET22b to construct a recombinant human neuregulin expression plasmid (PET22b-human neuregulin). This gene was expressed efficiently under the drive of T7 promoter. N-terminal of expressed gene was inserted at NdeI locus. C-terminal terminator is next to the last amino acid. The expressed protein did not form fusion protein with any amino acid. An 183bp fragment was obtained after endonuclease digestion analysis. The transformant was characterized by endonuclease digestion, and the double-stranded DNA was extracted for sequence analysis.

The cDNA sequence was found to be: AGC CAT CTT GTA AAA TGT GCG GAG AAG GAG AAA ACT TTC TGT GTG AAT GGA GGG GAG TGC TTC ATG GTG AAA GAC CTT TCA AAC CCC TCG AGA TAC TTG TGC AAG TGC CCA AAT GAG TTT ACT GGT GAT CGC TGC CAA AAC TAC GTA ATG GCC AGC TTC TAC AAG GCG GAG GAG CTG TAC CAG (SEQ ID. NO: 1). The deduced amino acid sequence based on the above cDNA sequence is: SHLVKCAEKEKTFCVNGGECFMVKDLSNPSRYLCKCPNEFTGDRCQNYVMASFYKAEEL YQ (SEQ ID. NO: 2).

After PCR amplification and endonuclease digestion analysis, a single colony of the engineering clone (BL21-PET22b-human-neuregulin) was randomly picked to inoculate in 2 ml LB-Amp liquid medium. The culture was incubated overnight at 37°C and shaking at 250 rpm. Then a proportion of pure culture was inoculated into 20 ml LB-Amp medium. The culture was collected after incubation at 37°C until the turbidity increased to 1.0 at OD_{600} and after IPTG was added for four hours to induce the expression. The inclusion body was collected after the cells were destructed.

After electrophoresis in 15% SDS-PAGE, thin-layer scanning analysis, Western-blotting, and repeated screening, an engineering strain (BL21-PET22b-human neuregulin) was characterized and established with stable high level expression of target protein neuregulin. The expressed target protein accounted for approximately 10% of the total protein in this strain. After high-pressure homogenate process, the target protein was presented in the form of inclusion body.

The engineering strain was analyzed by SDS-PAGE electrophoresis after four hours induction by IPTG. The inclusion body accounted for about 20% of the total proteins. Purified recombinant neuregulin specific activity was more than 5×10^3 EU/mg, indicating the construction of neuregulin producing strain was successful.

Example 3

Rat Model of ADM Cardiac Toxicity

One hundred forty male, SD rats (weight about 250 g, provided by the Department of Experimental Animals, previously Shanghai Medical University) were selected for experimental use. The animals were randomized into six groups: (1) ADM + vehicle (negative control); (2) normal rat; (3) ADM + neuregulin 233 ug/kg; (4) ADM + neuregulin 23.3 ug/kg; (5) ADM + neuregulin 2.33 ug/kg; and (6) ADM + neuregulin 0.233 ug/kg.

ADM was injected through the caudal vein once a week for four consecutive weeks. The dose was 3.3 mg/kg per injection. After the first injection of ADM, neuregulin or vehicle was administered once every three days through the caudal vein, with a total administration of nine times. The volume of dose was about 0.5 ml/100g.

The survival rate was calculated according to the number of dead animals every day. After five weeks, the survival rates were significantly higher in treatment groups than in negative control group. As shown in Figure 6, the survival rate in group of 23.3 ug/kg neuregulin was the highest.

Physiological comparisons were evaluated (*e.g.*, dp/dt, -dp/dt, LVP max/min, ECG). As shown in Figure 7, there were no significant difference between the normal group, positive control group, and treatment groups 4-6. However, the values in these groups were higher than the negative control group and Group 7. The difference between the negative control group and treatment group 7 was not statistically significant.

The pathological ratio of heart weight and body weight, thickness of ventricular wall, circumference of heart, microscopical evaluation of ventricular muscle section with HE staining were also evaluated. As shown in Figure 8, the ratios in treatment groups and control groups ranged from 0.00310 to 0.00313. No change was found in cardiac structure. The difference in ratios was not significant. As shown in Figure 9, the thickness of the left ventricular wall in treatment groups and control groups ranged from 1.79 to 1.99 mm. No significant change was identified in the thickness of left ventricular wall.

Moreover, the pathological score of myocardium was evaluated. Myocardial damage under light microscope included myocardial vacuolation, myofibril degeneration or coagulation necrosis. The damage was scored according to the range of lesion, ranging from no damage (0); mild damage, with lesion implicated in less than 10% of entire layer of myocardium (1); medium damage, with lesion involved in 10%-50% of entire layer of myocardium (2); severe damage, with the disease evident in more than 50% of the entire layer of myocardium (3). The size of cardiac muscle fiber was also calculated. Twenty fibers were counted on each section, and average value was compared. Difference between groups was compared by t-test.

Table 1 shows the pathological score of myocardium. The lower the pathological score, the better the efficacy. As shown in Table 1, the scores were significantly different between the negative control group, and treatment groups of 233 ug/kg, and 23.3 ug/kg neuregulin.

Table 1

	Normal	233 ug/kg	23.3 ug/kg	2.33 ug/kg	0.233 ug/kg	Vehicle(PBS)
Mean	0	1.5	1.83	2.5	2.5	2.83
SD	0	0	0.29	0.41	0	0.29

The above examples are included for illustrative purposes only and are not intended to limit the scope of the invention. Many variations to those described above are possible. Since modifications and variations to the examples described above will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

What is claimed is:

1. A method for preventing, treating or delaying cardiac toxicity in a mammal to which such prevention, treatment or delay is needed or desirable, comprising administering to a mammal *in vivo* an effective amount of a prophylactic or a therapeutic agent and an effective amount of: (i) a neuregulin protein or a functional fragment thereof; (ii) a nucleic acid encoding a neuregulin protein or a functional fragment thereof; or (iii) an agent that enhances production or function of said neuregulin, whereby said cardiac toxicity associated with administration of said prophylactic or therapeutic agent is prevented, treated or delayed.

2. The method of claim 1, wherein the prophylactic or therapeutic agent produces oxygen-derived free radicals which cause the cardiac toxicity.

3. The method of claim 1, wherein the prophylactic or therapeutic agent enhances lipid peroxidation which causes the cardiac toxicity.

4. The method of claim 1, wherein the cardiac toxicity to be prevented, treated or delayed is selected from the group consisting of tachycardia, arrhythmia and congestive heart failure.

5. The method of claim 1, wherein the cardiac toxicity to be prevented, treated or delayed is an acute or a chronic cardiac toxicity.

6. The method of claim 5, wherein the acute cardiac toxicity has a clinical feature selected from the group consisting of sinus tachycardia, arrhythmia, conduction block and ST-T segment alteration.

7. The method of claim 5, wherein the chronic cardiac toxicity has a clinical feature of irreversible congestive heart failure.

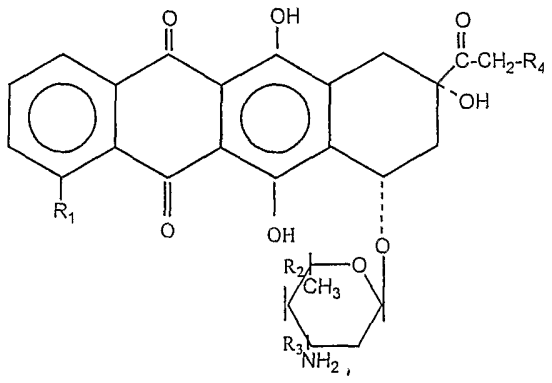
8. The method of claim 1, wherein the cardiac toxicity to be prevented, treated or delayed comprises a decrease in left ventricular ejection fraction (LVEF), a decrease in stroke volume (SV), a decrease in cardiac output (CO), or a decrease in cardiac index (CI).

9. The method of claim 1, wherein the cardiac toxicity to be prevented, treated or delayed comprises inhibition of the contracting and pumping ability of left ventricle.

10. The method of claim 1, wherein the prophylactic or therapeutic agent is selected from the group consisting of an anti-neoplasm agent, an antipsychotic agent, a tricyclic antidepressant, an interferon, an interleukin, and an anti-infectious agent.

11. The method of claim 10, wherein the anti-neoplasm agent is an anthracycline anti-neoplasm agent.

12. The method of claim 11, wherein the anthracycline anti-neoplasm agent has the following formula I:



wherein R1 is methoxy or hydrogen; and R2, R3 and R4 are hydroxy or hydrogen.

13. The method of claim 11, wherein the anthracycline anti-neoplasm agent is selected from the group consisting of adriamycin (or doxorubicin), daunorubicin, epirubicin, idarubicin, mitoxantrone, mitomycin, bleomycin, cyclophosphamide, fluorouracil, actinomycin D and vincristine.

14. The method of claim 13, wherein the anthracycline anti-neoplasm agent is adriamycin.

15. The method of claim 10, wherein the antipsychotic agent is selected from the group consisting of chlorpromazine, perphenazine and trifluperazine.

16. The method of claim 10, wherein the tricyclic antidepressant is selected from the group consisting of chlorimipramine, amitriptyline and doxepin.

17. The method of claim 10, wherein the interferon is interferon-α.

18. The method of claim 10, wherein the interleukin is interleukin-2.

19. The method of claim 10, wherein the anti-infectious agent is emetine.

20. The method of claim 1, wherein the neuregulin is selected from the group consisting of neuregulin 1, neuregulin 2, neuregulin 3, and neuregulin 4.

21. The method of claim 20, wherein the neuregulin 1 is neuregulin α2 or neuregulin β2.

22. The method of claim 21, wherein the neuregulin fragment is a neuregulin β2 comprising an amino acid sequence set forth in SEQ ID NO:2.

23. The method of claim 1, wherein the neuregulin protein or a functional fragment thereof, or a nucleic acid encoding a neuregulin protein or a functional fragment thereof, or an agent that enhances production or function of said neuregulin, is administered with a pharmaceutically acceptable carrier or excipient.

24. The method of claim 23, wherein a neuregulin protein, or a functional fragment thereof, is administered.

25. The method of claim 23, wherein a nucleic acid encoding a neuregulin protein, or a functional fragment thereof, is administered.

26. The method of claim 1, wherein the neuregulin protein or a functional fragment thereof, or a nucleic acid encoding a neuregulin protein or a functional fragment thereof, or an agent that enhances production or function of said neuregulin, is administered prior to, concurrently with, or subsequent to the administration of the prophylactic or therapeutic agent.

27. The method of claim 1, wherein the prophylactic or therapeutic agent is administered in an amount that is higher than a maximally allowed amount when the prophylactic or therapeutic agent is administered in the absence of the neuregulin protein or a functional fragment thereof, or a nucleic acid encoding a neuregulin protein or a functional fragment thereof, or an agent that enhances production or function of said neuregulin.

28. The method of claim 1, wherein the mammal is a human.

29. The method of claim 28, wherein the human has a malignant tumor selected from the group consisting of lung cancer, breast cancer, bladder carcinoma, testis carcinoma, thyroid cancer, soft tissue carcinoma, osteosarcoma, neuroblastoma, acute leukemia, malignant lymphoma, gastric carcinoma, liver cancer, esophageal carcinoma and cervical carcinoma.

FIGURE 1

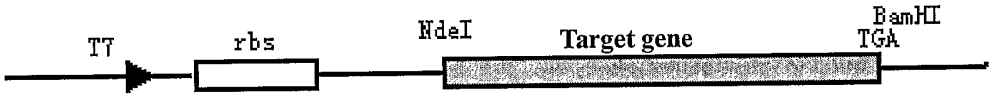


FIGURE 2

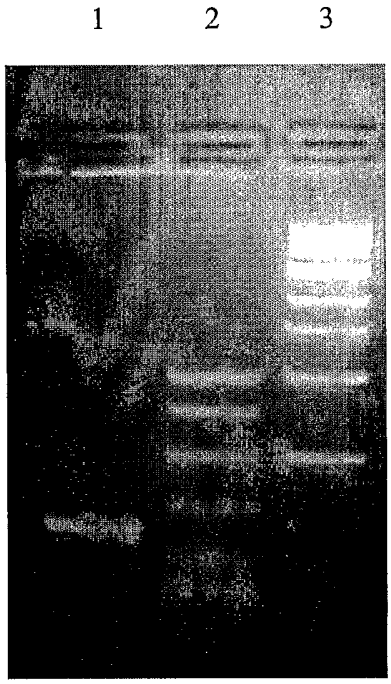


FIGURE 3

pET-22b(+) sequence landmarks	
T7 promoter	361-377
T7 transcription start	360
pelB coding sequence	224-289
Multiple cloning sites	
(*Nco* I - *Xho* I)	158-225
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	764-1843
pBR322 origin	3277
bla coding sequence	4038-4895
f1 origin	5027-5482

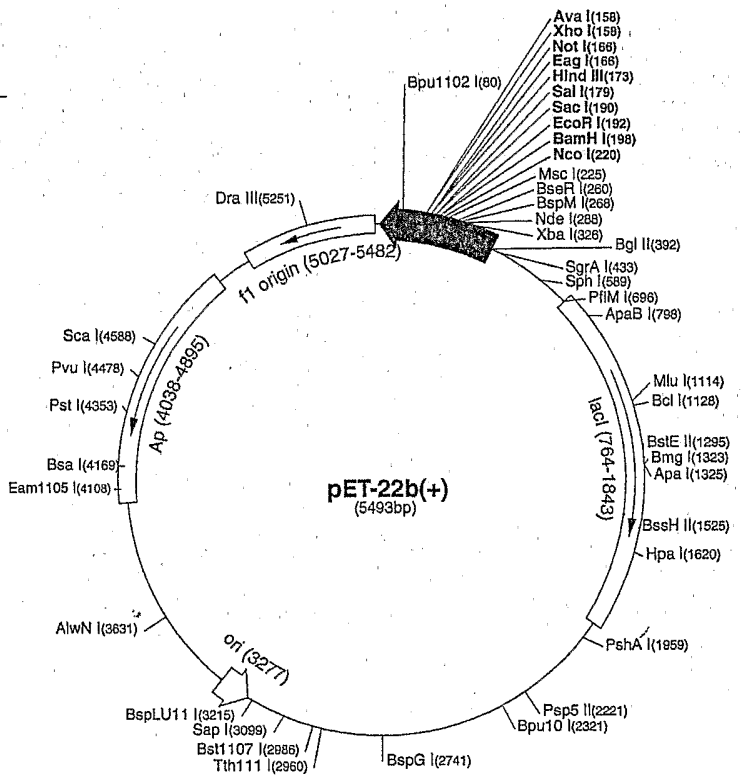


FIGURE 4

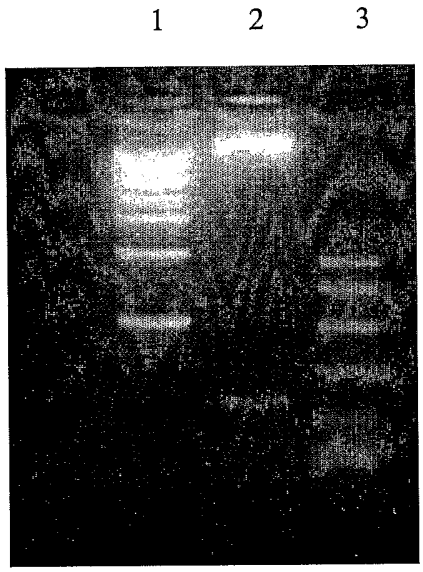


FIGURE 5

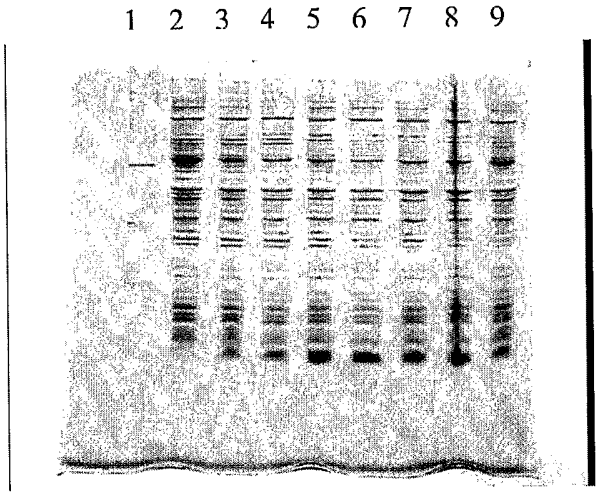


FIGURE 6

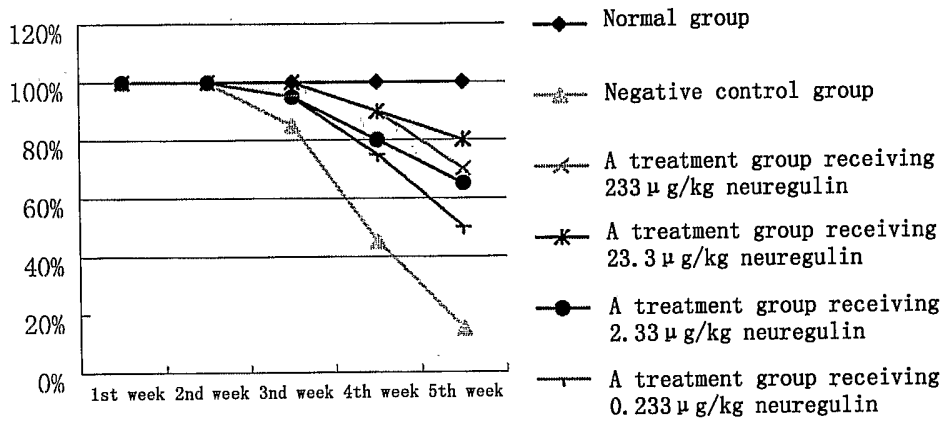

120%
100%
80%
60%
40%
20%
0%
1st week 2nd week 3nd week 4th week 5th week
Normal group
Negative control group
A treatment group receiving 233 μg/kg neuregulin
A treatment group receiving 23.3 μg/kg neuregulin
A treatment group receiving 2.33 μg/kg neuregulin
A treatment group receiving 0.233 μg/kg neuregulin

FIGURE 7

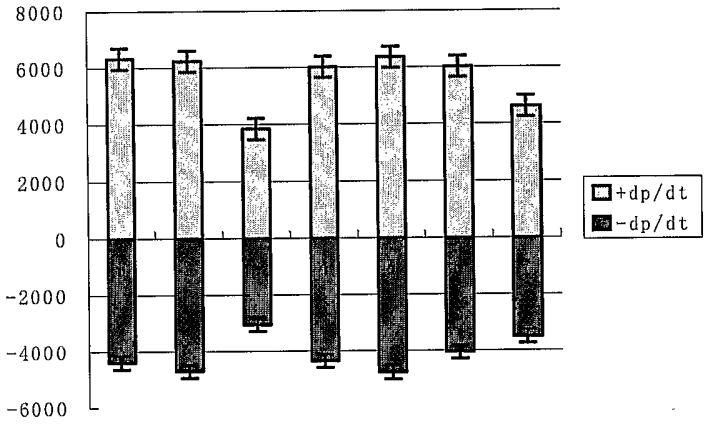


FIGURE 8

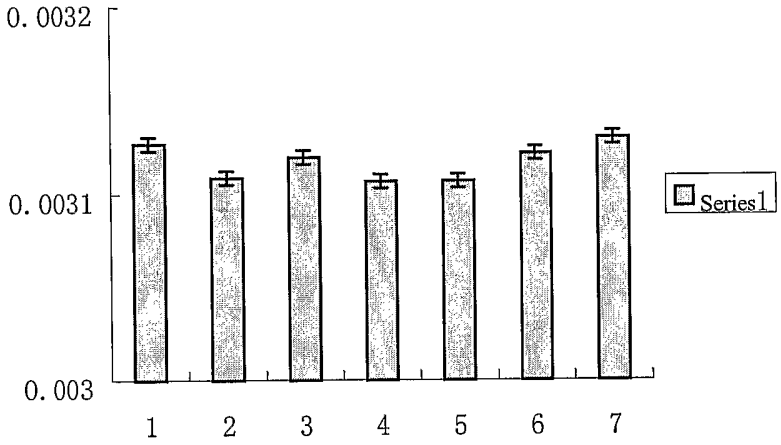
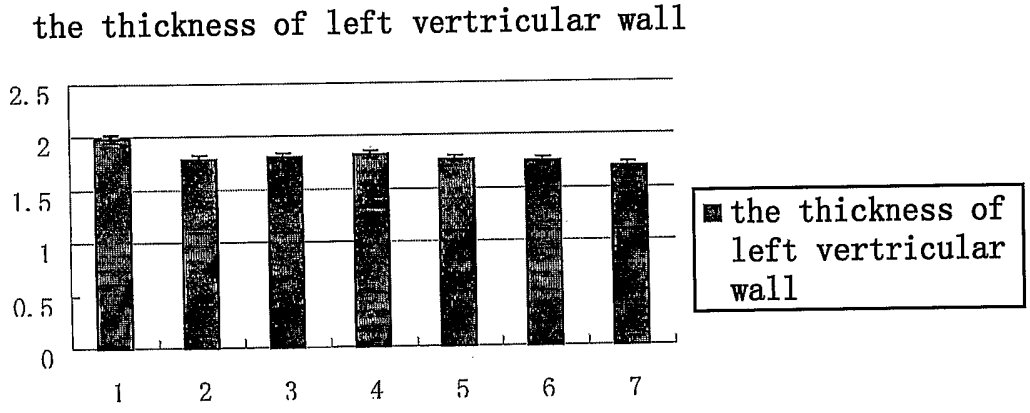


FIGURE 9


the thickness of left vertricular wall
2.5
2
1.5
1
0.5
0
1
2
3
4
5
6
7
the thickness of left vertricular wall

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN02/00664

A. CLASSIFICATION OF SUBJECT MATTER

A61K38/19 ; A61K48/00 ; A61P9/00; A61K0/00

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)

A61K38/19 ; A61K48/00 ; A61P9/00; A61K0/00

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)

CNPAT, EPOQUE(WPI), NCBI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A2, 0064400, ((BETH-N) BETH ISRAEL DEACONESS MEDICAL CENT et al), 02, November, 2000, see the entire document.	1-29
A	WO,A1, 0037095, ((CHAN-N) CHANG CARDIAC RES INST VICTOR), 29, June, 2000, see the entire document.	1-29

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"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)

"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

"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

"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

"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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 May 2003(26.05.2003)	12 JUN 2003 (12.06.03)
Name and mailing address of the ISA/CN 6 Xitucheng Rd., Jimen Bridge, Haidian District, 100088 Beijing, China Facsimile No. 86-10-62019451	Authorized officer Wei Chunbao Telephone No. 86-10-62093435

Form PCT/ISA /210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN02/00684

Patent document cited in search report	Publication date	Patent family members	Publication date
WO-A2-0064400	02-11-2000	AU-A-200049744	10-11-2000
WO-A1-0037095	29-06-2000	EP-A1-1158998 AU-A-200024224	05-12-2001 12-07-2000