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Peptides effective in the treatment of tumors and other conditions requiring the removal or destruction of cells

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**PEPTIDES EFFECTIVE IN THE TREATMENT OF TUMORS AND
OTHER CONDITIONS REQUIRING THE REMOVAL OR
DESTRUCTION OF CELLS**

5 This application claims priority to provisional application Serial No.
60/306,161, entitled: "Proteins and peptides effective in the treatment of tumors
and other conditions requiring the removal or destruction of cells," filed on July 19,
2001, provisional patent application Serial No. 60/306,150, entitled: "Peptides
effective in the treatment of tumors and other conditions requiring the removal or
10 destruction of cells," filed July 19, 2001, and provisional patent application Serial
No. 60/331,477, entitled: "Peptides effective in the treatment of tumors and other
conditions requiring the removal or destruction of cells," filed on November 16,
2001, the disclosure of each of which is incorporated by reference herein in its
entirety.

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FIELD OF THE INVENTION

 The present invention is directed to methods of treating conditions requiring
removal or destruction of cellular elements, such as benign or malignant tumors in
humans, using compounds based on peptides comprising amino acid sequences
20 corresponding to, similar to or homologous to part of the amino acid sequence of
neural thread proteins. The method includes, but is not limited to, administering
the compounds intramuscularly, orally, intravenously, intrathecally, intratumorally,
intranasally, topically, transdermally, etc., either alone or conjugated to a carrier.

25

BACKGROUND OF THE INVENTION

 The essence of many medical treatments and procedures involves the
removal or destruction of harmful or unwanted tissue. Examples of such important
treatments include the surgical removal of cancerous growths, the destruction of
metastatic tumors through chemotherapy, and the reduction of glandular (e.g.
30 prostate) hyperplasia. Other examples include the removal of unwanted facial hair,
warts, subcutaneous tissue, lymphoid tissue or fatty tissue.

There is a need for an effective agent that will destroy and hence either facilitate the removal of or inhibit the further growth of harmful or unwanted cells and tissue, but that will have mainly local effects and minimal or absent systemic toxicity. Neural thread proteins and their related molecules are one class of such agents as disclosed in pending United States patent application Serial No. 10/092,934, entitled: *Methods of Treating Tumors and Related Conditions Using Neural Thread Proteins*, filed on March 8, 2002, the disclosure of which is incorporated by reference herein in its entirety. Peptides containing amino acid sequences corresponding to part of the amino acid sequence of a neural thread protein, AD7c-NTP also are such agents. These peptides are disclosed in pending United States application Serial No. 10/153,334, entitled: *Peptides Effective in the Treatment of Tumors and Other Conditions Requiring the Removal or Destruction of Cells*, filed May 24, 2002, the disclosure of which is incorporated by reference herein in its entirety.

Disclosed herein are certain other fragments of neural thread proteins that also are useful in treating tumors and other conditions requiring removal or destruction of cells.

Cancer is an abnormality in a cell's internal regulatory mechanisms that results in uncontrolled growth and reproduction of the cell. Normal cells make up tissues, and when these cells lose their ability to behave as a specified, controlled, and coordinated unit (dedifferentiation) the defect leads to disarray amongst the cell population. When this occurs, a tumor is formed.

Benign overgrowths of tissue are abnormalities in which it is desirable to remove cells from an organism. Benign tumors are cellular proliferations that do not metastasize throughout the body but do, however, cause disease symptoms. Such tumors can be lethal if they are located in inaccessible areas in organs such as the brain. There are benign tumors of organs including lung, brain, skin, pituitary, thyroid, adrenal cortex and medulla, ovary, uterus, testis, connective tissue, muscle, intestines, ear, nose, throat, tonsils, mouth, liver, gall bladder, pancreas, prostate, heart, and other organs.

Surgery often is the first step in the treatment of cancer. The objective of surgery varies. Sometimes it is used to remove as much of the evident tumor as possible, or at least to debulk it (remove the major bulk(s) of tumor so that there is less that needs to be treated by other means). Depending on the cancer type and location, surgery may also provide some symptomatic relief to the patient. For instance, if a surgeon can remove a large portion of an expanding brain tumor, the pressure inside the skull will decrease, leading to improvement in the patient's symptoms.

Not all tumors are amenable to surgery. Some may be located in parts of the body that make them impossible to completely remove. Examples of these would be tumors in the brainstem (a part of the brain that controls breathing) or a tumor which has grown in and around a major blood vessel. In these cases, the role of surgery is limited due to the high risk associated with tumor removal.

In some cases, surgery is not used to debulk tumor because it is not necessary. An example is Hodgkin's lymphoma, a cancer of the lymph nodes that responds very well to combinations of chemotherapy and radiation therapy. In Hodgkin's lymphoma, surgery is rarely needed to achieve cure, but almost always used to establish a diagnosis.

Chemotherapy is a common form of cancer treatment that involves the use of medications (usually given by mouth or injection) which specifically attack rapidly dividing cells (such as those found in a tumor) throughout the body. This makes chemotherapy useful in treating cancers that have already metastasized, as well as tumors that have a high chance of spreading through the blood and lymphatic systems but are not evident beyond the primary tumor. Chemotherapy may also be used to enhance the response of localized tumors to surgery and radiation therapy. This is the case, for example, for some cancers of the head and neck.

Unfortunately, other cells in the human body that also normally divide rapidly (such as the lining of the stomach and hair) also are affected by chemotherapy. For this reason, many chemotherapy agents induce undesirable side effects such as nausea, vomiting, anemia, hair loss or other symptoms. These side

effects are temporary, and there exist medications that can help alleviate many of these side effects. As our knowledge has continued to grow, researchers have devised newer chemotherapeutic agents that are not only better at killing cancer cells, but that also have fewer side effects for the patient.

5 Chemotherapy is administered to patients in a variety of ways. Some are pills and some are administered by an intravenous or other injection. For injectable chemotherapy, a patient goes to the doctor's office or hospital for treatment. Other chemotherapeutic agents require continuous infusion into the bloodstream, 24 hours a day. For these types of chemotherapy, a minor surgical procedure may be performed to implant a small pump worn by the patient. The pump then slowly
10 administers the medication. In many cases, a permanent port is placed in a patient's vein to eliminate the requirement of repeated needle sticks.

 Radiation therapy is another commonly used weapon in the fight against cancer. Radiation kills cancer by damaging the DNA within the tumor cells. The
15 radiation is delivered in different ways. The most common involves pointing a beam of radiation at the patient in a highly precise manner, focusing on the tumor. To do this, a patient lies on a table and the beam moves around him/her. The procedure lasts minutes, but may be done daily for several weeks (depending on the type of tumor), to achieve a particular total prescribed dose.

20 Another radiation method sometimes employed, called brachytherapy, involves taking radioactive pellets (seeds) or wires and implanting them in the body in the area of the tumor. The implants can be temporary or permanent. For permanent implants, the radiation in the seeds decays over a period of days or weeks so that the patient is not radioactive. For temporary implants, the entire dose
25 of radiation is usually delivered in a few days, and the patient must remain in the hospital during that time. For both types of brachytherapy, radiation is generally delivered to a very targeted area to gain local control over a cancer (as opposed to treating the whole body, as chemotherapy does.)

 Some highly selected patients may be referred for bone marrow transplants.
30 This procedure is usually performed either because a patient has a cancer that is particularly aggressive or because they have a cancer that has relapsed after being

treated with conventional therapy. Bone marrow transplantation is a complicated procedure. There are many types, and they vary in their potential for causing side effects and cure. Most transplants are performed at special centers, and in many cases their use is considered investigational.

- 5 There are a number of other therapies, though most of them are still being explored in clinical trials and have not yet become standard care. Examples include the use of immunotherapy, monoclonal antibodies, anti-angiogenesis factors, and gene therapy.

- 10 Immunotherapy: There are various techniques designed to help the patient's own immune system fight the cancer, quite separately from radiation or chemotherapy. Oftentimes, to achieve the goal researchers inject the patient with a specially derived vaccine.

- 15 Monoclonal Antibodies: These are antibodies designed to attach to cancerous cells (and not normal cells) by taking advantage of differences between cancerous and non-cancerous cells in their antigenic and/or other characteristics. The antibodies can be administered to the patient alone or conjugated to various cytotoxic compounds or in radioactive form, such that the antibody preferentially targets the cancerous cells, thereby delivering the toxic agent or radioactivity to the desired cells.

- 20 Anti-Angiogenesis Factors: As cancer cells rapidly divide and tumors grow, they can soon outgrow their blood supply. To compensate for this, some tumors secrete a substance believed to help induce the growth of blood vessels in their vicinity, thus providing the cancer cells with a vascular source of nutrients. Experimental therapies have been designed to arrest the growth of blood vessels to
25 tumors.

- Gene Therapy: Cancer is the product of a series of mutations that ultimately lead to the production of a cancer cell and its excessive proliferation. Cancers can be treated by introducing genes to the cancer cells that will act either to check or stop the cancer's proliferation, turn on the cell's programmed cell
30 mechanisms to destroy the cell, enhance immune recognition of the cell, or express

a pro-drug that converts to a toxic metabolite or a cytokine that inhibits tumor growth.

Benign tumors and malformations also can be treated by a variety of methods including surgery, radiotherapy, drug therapy, thermal or electric ablation, cryotherapy, and others. Although benign tumors do not metastasize, they can grow large and they can recur. Surgical extirpation of benign tumors has all the difficulties and side effects of surgery in general and oftentimes must be repeatedly performed for some benign tumors, such as for pituitary adenomas, meningiomas of the brain, prostatic hyperplasia, and others.

There are still other conditions involving unwanted cellular elements where selective cellular removal is desirable. For example, heart disease and strokes are commonly caused by atherosclerosis, which is a proliferative lesion of fibrofatty and modified smooth muscle elements that distort the blood vessel wall, narrow the lumen, constrict blood flow, predispose to focal blood clots, and ultimately lead to blockage and infarction. Various treatments for atherosclerosis include bypass grafts; artificial grafts; angioplasty with recanalization, curettage, radiation, laser, or other removal; pharmacotherapy to inhibit atherosclerosis through lipid reduction; anti-clotting therapies; and general measures of diet, exercise, and lifestyle. A method for removing atherosclerotic lesions without the risk and side effects of surgical procedures is needed.

Other examples of unwanted cellular elements where selective cellular removal is desirable include viral induced growths, such as warts. Another example is hypertrophic inflammatory masses found in inflammatory conditions, and hypertrophic scars or keloids. Still other examples are found in cosmetic contexts such as the removal of unwanted hair, e.g., facial hair, or for shrinkage of unwanted tissue areas for cosmetic purposes, such as in the facial dermis and connective tissues or in the dermas and connective tissue of the extremities.

Still other examples will become evident to those of ordinary skill in the art upon reading the disclosure herein. In all or most of these examples, there is a need for treatments that can remove or destroy the unwanted cellular elements

without the risks and side effects of conventional therapies, or to remove the unwanted cellular elements with more precision.

Neural thread proteins (NTP) are a family of recently characterized brain proteins. One member of this family, AD7c-NTP, is a ~41 kD membrane
5 associated phosphoprotein with functions associated with neuritic sprouting (de la Monte *et al.*, *J. Clin. Invest.*, 100:3093-3104 (1997); de la Monte *et al.*, *Alz. Rep.*, 2:327-332 (1999); de la Monte SM and Wands JR, *Journal of Alzheimer's Disease*, 3:345-353 (2001)). The gene that encodes AD7c-NTP and predicted protein sequence for AD7c-NTP has been identified and described (de la Monte *et al.*, *J.*
10 *Clin. Invest.*, 100:3093-3104 (1997)). In addition to the ~41 kD species, other species of neural thread protein (~26 kD, ~21 kD, ~17 kD, and ~15 kD) have been identified and associated with neuroectodermal tumors, astrocytomas, and glioblastomas and with injury due to hypoxia, schema, or cerebral infarction (Xu *et al.*, *Cancer Research*, 53:3823-3829 (1993); de la Monte *et al.*, *J. Neuropathol.*
15 *Exp. Neurol.*, 55(10):1038-50 (1996), de la Monte *et al.*, *J. Neurol. Sci.*, 138(1-2):26-35 (1996); de la Monte *et al.*, *J. Neurol. Sci.*, 135(2):118-25 (1996); de la Monte *et al.*, *J. Clin. Invest.*, 100:3093-3104 (1997); and de la Monte *et al.*, *Alz. Rep.*, 2:327-332 (1999)).

Species of neural thread protein have been described and claimed in U.S.
20 Patent Nos. 5,948,634; 5,948,888; and 5,830,670, all for "Neural Thread Protein Gene Expression and Detection of Alzheimer's Disease" and in U.S. Patent No. 6,071,705 for "Method of Detecting Neurological Disease or Dysfunction." The disclosures of these patents are specifically incorporated herein by reference in their entirety. As described therein, NTP is upregulated and produced during cell
25 death. Thus, dead and dying nerve cells are described as overproducing NTP, and accordingly, its presence indicates the death of nerve cells and the onset of Alzheimer's disease (AD).

Other species of neural thread protein have been identified as other products of the AD7c-NTP gene (e.g. a 112 amino acid protein described in NCBI Entrez-
30 Protein database Accession #XP_032307 PID g15928971) or as being similar to neural thread proteins (e.g. a 106 amino acid protein described in NCBI Entrez-

Protein database Accession #AAH14951 PID g15928971, another 106 amino acid protein described in NCBI Entrez-Protein database Accession #XP_039102 PID g18599339 and a 61 amino acid protein described in NCBI Entrez-Protein database Accession #AAH02534 PID g12803421).

- 5 Neural thread protein is associated with AD and NTP is upregulated in association with cell death in AD. AD7c-NTP mRNA is upregulated in AD brain compared to controls; AD7c-NTP protein levels in brain and in CSF are higher in AD than controls; and AD7c-NTP immunoreactivity is found in senile plaques, in neurofibrillary tangles (NFT), in degenerating neurons, neuropil threads, and
- 10 dystrophic neurotic sprouts in AD and Down syndrome brains (Ozturk *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:419-423 (1989); de la Monte *et al.*, *J. Clin. Invest.*, 86(3):1004-13 (1990); de la Monte *et al.*, *J. Neurol. Sci.*, 113(2):152-64 (1992); de la Monte *et al.*, *Ann. Neurol.*, 32(6):733-42 (1992); de la Monte *et al.*, *J. Neuropathol. Exp. Neurol.*, 55(10):1038-50 (1996); de la Monte *et al.*, *J. Neurol. Sci.*, 138(1-2):26-35 (1996); de la Monte *et al.*, *J. Neurol. Sci.*, 135(2):118-25 (1996); de la Monte *et al.*, *J. Clin. Invest.*, 100:3093-3104 (1997); and de la Monte *et al.*, *Alz. Rep.*, 2:327-332 (1999)). NTP is localized within cells, within fine processes within the neuropil, or is extracellular in both AD and Down's Syndrome brains. de la Monte *et al.*, *Ann. Neurol.*, 32(6):733-42 (1992).
- 20 Elevated levels of AD7c-NTP protein have been found in both CSF and urine of AD patients (de la Monte and Wands, *Front Biosci* 7: 989-96 (2002); de la Monte and Wands, *Journal of Alzheimer's Disease* 3: 345-353 (2001); Munzar *et al.*, *Alzheimer's Reports* 4: 61-65 (2001); Kahle *et al.*, *Neurology* 54: 1498-1504 (2000); Munzar *et al.*, *Alzheimer Reports* 3: 155-159 (2000); de la Monte *et al.*, *Alzheimer's Reports* 2: 327-332 (1999); and de la Monte *et al.*, *J Clin Invest* 100: 3093-3104 (1997).
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Over-expression of NTP also has been linked to the process of cell death in Alzheimer's disease (de la Monte and Wands, *J. Neuropathol. Exp. Neurol.*, 60:195-207 (2001); de la Monte and Wands, *Cell Mol Life Sci* 58: 844-49 (2001).

- 30 AD7c-NTP has also been identified in Down's Syndrome brain tissue (Wands *et al.*, International Patent Publication No. WO 90/06993; de la Monte *et al.*, *J Neurol*

Sci 135:118-25 (1996); de la Monte *et al.*, *Alz. Rep.*, 2:327-332 (1999)). There is some evidence that over-expression of NTP also may be associated with normal tension glaucoma (Golubnitschaja-Labudova *et al*, *Curr Eye Res* 21:867-76(2000)).

NTP has proven to be an effective agent for causing cell death both *in vitro* in glioma and neuroblastoma cell cultures and *in vivo* in normal rodent muscle tissue, subcutaneous connective tissue, and dermis, and in a variety of different human and non-human origin tumors, including mammary carcinoma, skin carcinoma and papilloma, colon carcinoma, glioma of brain, and others in rodent models. See the pending United States patent application Serial No. 10/092,934, *Methods of Treating Tumors and Related Conditions Using Neural Thread Proteins*, filed on March 8, 2002.

Certain peptide sequences of AD7c-NTP ("AD7c-NTP peptides") have also proven to be effective agents for causing cell death both *in vitro* in glioma and neuroblastoma cell cultures and/or *in vivo* in normal rodent muscle tissue, subcutaneous connective tissue, dermis and other tissue. See United States patent application Serial No. 10/153,334, entitled: *Peptides Effective in the Treatment of Tumors and Other Conditions Requiring the Removal or Destruction of Cells*, filed on May 24, 2002.

Throughout this description, including the foregoing description of related art, any and all publicly available documents described herein, including any and all U. S. patents, are specifically incorporated by reference herein in their entirety. The foregoing description of related art is not intended in any way as an admission that any of the documents described therein, including pending United States patent applications, are prior art to the present invention or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

There remains a need in the art for new, less toxic treatments for treating unwanted cellular elements. The present invention satisfies these needs.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

SUMMARY OF THE INVENTION

The present invention is directed to peptides, compositions, and methods of treating unwanted cellular proliferations, such as benign and malignant tumors, glandular (e.g. prostate) hyperplasia, unwanted facial hair, warts, and unwanted fatty

tissue. Such a method comprises administering to a mammal in need a therapeutically effective amount of a NTP Peptide known to be an effective agent for causing cell death. The expression NTP Peptide is defined below.

The peptides of the present invention have at least one amino acid sequence corresponding to part of the amino acid sequence of a species of neural thread protein. The compositions of the invention include the peptides and a pharmaceutically acceptable carrier, or the peptides conjugated to a carrier, or the like, or the peptides encapsulated within a polymeric matrix, etc.

The present invention involves in part the discovery that peptide sequences contained in AD7c-NTP that the inventors have found to be effective agents for the destruction or removal of harmful or unwanted cells, and variants and homologs thereof, also are found in other proteins in other organisms, including humans and other mammals. Once the peptide sequences have been discovered, these proteins can be found by a person ordinarily skilled in the art through the use of widely available public and commercial protein databases such as the National Center Biotechnology Information's Protein database and search programs such as BLAST® (Basic Local Alignment Search Tool). *See* Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", *Nucleic Acids Res.* 25:3389-3402.

Accordingly, the present invention provides a peptide comprising at least one peptide selected from group consisting of:

- a) The peptide represented by the amino acid sequence in SEQ ID NO. 10 (Pro-Gly-Phe-Phe-Lys-Leu-Phe-Ser-Cys-Pro-Ser-Leu-Leu-Ser-Ser-Trp-Asp-Tyr-Arg-Arg)
- b) The peptide represented by the amino acid sequence in SEQ ID NO. 22 (Ser-Ser-Trp-Asp-Tyr-Arg-Arg-Phe-Ile-Leu-Phe-Phe-Leu);
- c) The peptide represented by the amino acid sequence in SEQ ID NO. 23 (Trp-Asp-Tyr-Arg-Arg-Phe-Ile-Phe-Asn-Phe-Leu);
- d) The peptide represented by the amino acid sequence in SEQ ID NO. 24 (Phe-Asn-Phe-Cys-Leu-Phe);
- e) The peptide represented by the amino acid sequence in SEQ ID NO. 25 (Phe-Ile-Phe-Asn-Phe-Leu); and
- f) The peptide represented by the amino acid sequence in SEQ ID NO. 26 (Pro-Ala-Ser-Ala-Ser-Pro-Val-Ala-Gly-Ile-Thr-Gly-Met);

wherein said peptide is effective as an agent for the destruction or removal of unwanted or harmful cells.

The present invention further provides a peptide consisting of at least one peptide selected from:

- 5 a) the peptide represented by the amino acid sequence in SEQ ID NO. 19 (Leu-Pro-Ser-Ser-Trp-Asp-Tyr-Arg-Arg); and
- b) the peptide represented by the amino acid sequence in SEQ ID NO. 28 (Pro-Ala-Ser-Ala-Ser-Gln-Ser-Ala-Gly-Ile-Thr-Gly-Val);

10 wherein said peptide is effective as an agent for the destruction or removal of unwanted or harmful cells.

A person having ordinary skill in the art then can screen these proteins by using the assay method described herein to determine their effectiveness as agents for the destruction or removal of unwanted or harmful cells. A person ordinarily skilled in the art, having found one or more such effective agents, then can determine which portions
15 of those agents contain sequences homologous with or similar to the AD7c-NTP peptide sequences described herein, or described in pending United States application Serial No. 10/153,334, entitled: *Peptides Effective in the Treatment of Tumors and Other Conditions Requiring the Removal or Destruction of Cells*, filed May 24, 2002, have those portions of those agents synthesized using methods known to those skilled
20 in the art, and test the synthesized agents for their effectiveness as agents for the destruction or removal of unwanted or harmful cells. Furthermore, a person ordinarily skilled in the art could also use the amino acid sequences of any such proteins found to determine other peptide sequences not similar to or homologous with homologous with or similar to the AD7c-NTP peptide sequences described herein, and described in
25 pending United States application Serial No. 10/153,334, entitled: *Peptides Effective in the Treatment of Tumors and Other Conditions Requiring the Removal or Destruction of Cells*, filed May 24, 2002. These new synthesized sequences can then be tested for their effectiveness as agents for the destruction or removal of unwanted or harmful cells.

30 The present invention further provides a peptide comprising a homologue, derivative, fragment or variant of the peptide according to the invention wherein the homologue, derivative, fragment or variant is effective as an agent for the destruction or removal of unwanted or harmful cells.

The present invention further provides a peptide comprising an amino acid in a reverse-D order based on the amino acid sequence for the peptide according to the invention.

5 The present invention further provides a peptide comprising a peptide according to the invention modified by from one to 25 additional amino acids flanking either the 3' or 5' end of the peptide.

The present invention further provides a peptide comprising at least two peptides according to the invention.

10 The present invention further provides a peptide comprising at least two repetitions of the peptide according to the invention.

The present invention further provides a mimetic of a peptide according to the invention.

15 The present invention further provides a peptide comprising a peptide according to the invention fused to an antibody, fragment of an antibody or an antibody-like molecule.

The present invention further provides a method of treating a condition in a patient requiring removal or destruction of cells comprising administering to a mammal in need a therapeutically effective amount of a peptide according to the invention.

20 The inventive peptides or proteins ("cell death peptide") can be administered alone or conjugated to a carrier or an antibody. The cell death peptide can be administered intramuscularly, orally, intravenously, intraperitoneally, intracerebrally (intraparenchymally), intracerebroventricularly, intratumorally, intralesionally, intradermally, intrathecally, intranasally, intraocularly, intraarterially, topically, transdermally, via an aerosol, infusion, bolus injection, implantation device, sustained
25 release system etc., either alone or conjugated to a carrier. Alternatively, the cell death peptide can be expressed *in vivo* by administering a gene that expresses the peptide, by administering a vaccine that induces such production or by introducing cells, bacteria or viruses that express the peptide *in vivo*, either because of genetic modification or otherwise.

30 The present invention further provides a nucleic acid encoding an amino acid sequence corresponding to a peptide according to the invention and homologues, fragments and variants thereof wherein the homologues, fragments and variants are effective as agents for the destruction or removal of unwanted or harmful cells.

35 The present invention further provides a composition comprising one or more nucleic acids according to the invention and a pharmaceutically acceptable carrier therefor.

The present invention further provides use of a peptide according to the invention for the manufacture of a medicament for treating a condition in a patient requiring removal or destruction of cells.

- 5 In addition, the cell death peptide may be used in conjunction with other therapies for treating benign and malignant tumors and other unwanted or harmful cellular growths. Both the foregoing general description and the following detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following detailed
- 10 description of the invention.

BRIEF DESCRIPTION OF THE FIGURES

- Figure 1: Shows the complete amino acid sequence and nucleic acid sequence of the AD7c-NTP gene and the AD7c-NTP protein product of that gene (Sequences 120 and 121 from U.S. Patent Nos. 5,830,670, 5,948,634, and 5,948,888; de la Monte *et al.*, *J. Clin. Invest.*, 100:3093-3104 (1997); NCBI Entrez-Protein Accession # AAC08737; PID g3002527) [SEQ ID NO. 1].
- Figure 2: Shows the complete amino acid sequences of the 122 amino acid neural thread protein (Sequence 40 from U.S. Patent Nos. 5,830,670, 5,948,634, and 5,948,888; NCBI Entrez-Protein Accession #AAE25447 PID g10048540) [SEQ ID NO. 2].
- Figure 3: Shows the complete amino acid sequences of the 112 amino acid neural thread protein (NCBI Entrez-Protein Accession #XP_032307 PID g15928971) [SEQ ID NO. 3].
- Figure 4: Shows the complete amino acid sequences of a 106 amino acid neural thread protein-like protein (NCBI Entrez-Protein Accession #AAH14951 PID g15928971) [SEQ ID NO. 4].
- Figure 5: Shows the complete amino acid sequences of a 106 amino acid neural thread protein-like protein (NCBI Entrez-Protein Accession #XP_039102 PID g18599339) [SEQ ID NO. 5].
- Figure 6: Shows the complete amino acid sequences of the 98 amino acid neural thread protein (Sequence 30 from U.S. Patent Nos. 5,830,670, 5,948,634, and 5,948,888; NCBI Entrez-Protein Accession #AAE25445, PID g10048538) [SEQ ID NO. 6].
- Figure 7: Shows the complete amino acid sequences of the 75 amino acid neural thread protein (Sequence 48 from U.S. Patent Nos. 5,830,670, 5,948,634, and 5,948,888; NCBI Entrez-Protein Accession #AAE25448, PID g10048541) [SEQ ID NO. 7].
- Figure 8: Shows the complete amino acid sequences of the 68 amino acid neural thread protein (Sequence 36 from U.S. Patent Nos.

5,830,670, 5,948,634, and 5,948,888; NCBI Entrez-Protein
 Accession #AAE25446, PID g10048539) [SEQ ID NO. 8].
 Figure 9: Shows the complete amino acid sequences of the 61 amino acid
 neural thread protein-like protein (NCBI Entrez-Protein Accession
 #AAH02534, PID g12803421) [SEQ ID NO. 9].

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Terms and phrases used herein are defined as set forth below unless
 otherwise specified.

10 The expression "AD7c-NTP" refers to the ~41kD protein and the gene and
 the nucleic acid sequences coding for it described in de la Monte et al., *J. Clin.*
Invest., 100:3093-104 (1997), in Sequences 120 and 121 of U.S. Patent Nos.
 5,948,634, 5,948,888, and 5,830,670 and in GenBank #AF010144, the nucleic acid
 and amino acid sequences for which are illustrated in Figure 1. The term "AD7c-
 15 NTP" also includes biologically active fragments, variants, derivatives,
 homologues and mimetics of AD7c-NTP.

The term "NTP" or "neural thread protein" refers to neural thread proteins
 and related molecules (including pancreatic thread protein) and the nucleic acid
 sequences coding for those proteins, and includes (but is not limited to) the
 20 following proteins and the nucleic acid sequences encoding the amino acid
 sequences for these proteins:

- (a) AD7c-NTP;
- (b) the ~42, ~26, ~21, ~17, ~14, and ~8 kD species of neural thread
 protein as described in U.S. Patent Nos. 5,948,634, 5,948,888,
 25 5,830,670, and 6,071,705 and in de la Monte et al., *J. Neuropathol.*
Exp. Neurol., 55(10):1038-50 (1996), de la Monte et al., *J. Neurol.*
Sci., 138(1-2):26-35 (1996); de la Monte et al., *J. Neurol. Sci.*,
 135(2):118-25 (1996), de la Monte et al., *J. Clin. Invest.*,
 100:3093-3104 (1997) and de la Monte et al., *Alz. Rep.*, 2:327-
 30 332 (1999);

- (c) proteins specifically recognized by monoclonal antibody #2 on deposit with the American Type Culture Collection, Manassas, Va., under accession number HB-12546 or monoclonal antibody #5 on deposit with the American Type Culture Collection, Manassas, Va., under accession number HB-12545;
- (d) proteins coded by the AD7c-NTP gene;
- (e) the 122 amino acid neural thread protein described in Sequence 40 from U.S. Patent Nos. 5,830,670, 5,948,634, and 5,948,888 and listed in NCBI Entrez-Protein Accession #AAE25447, PID g10048540, the amino acid sequences for which is illustrated in Figure 2;
- (f) the 112 amino acid neural thread protein listed in NCBI Entrez-Protein Accession #XP_032307, PID g14725132, the amino acid sequences for which is illustrated in Figure 3;
- (g) a 106 amino acid neural thread protein-like protein listed in NCBI Entrez-Protein Accession #AAH14951 PID g15928971, the amino acid sequences for which is illustrated in Figure 4;
- (h) a 106 amino acid neural thread protein-like protein listed in NCBI Entrez-Protein Accession #XP_039102, PID g18599339, the amino acid sequence for which is illustrated in Figure 5;
- (i) the 98 amino acid neural thread protein described in Sequence 30 from U.S. Patent Nos. 5,830,670, 5,948,634, and 5,948,888 and listed in NCBI Entrez-Protein Accession # AAE25445, PID g10048538, the amino acid sequences for which is illustrated in Figure 6;
- (j) the 75 amino acid neural thread protein described in Sequence 48 from U.S. Patent Nos. 5,830,670, 5,948,634, and 5,948,888 and listed in NCBI Entrez-Protein Accession #AAE25448, PID g10048541, the amino acid sequences for which is illustrated in Figure 7;

- (k) the 68 amino acid neural thread protein described in Sequence 36 from U.S. Patent Nos. 5,830,670, 5,948,634, and 5,948,888 and listed in NCBI Entrez-Protein Accession #AAE25446, PID g10048539, the amino acid sequences for which is illustrated in Figure 8;
- (l) the 61 amino acid neural thread protein-like protein listed in NCBI Entrez-Protein Accession #AAH02534, PID g12803421, the amino acid sequences for which is illustrated in Figure 9;
- (m) pancreatic thread protein;
- (n) the neural pancreatic thread protein (NPTP) described in U.S. Patent No. 6,071,705; and
- (o) proteins specifically recognized by the antibodies produced by a hybridoma from the group consisting of HB 9934, HB 9935, and HB 9936 deposited at the American Type Culture Collection.
- The expression "NTP peptide" refers to peptides comprising amino acid sequences corresponding to at least a part of the amino acid sequence of NTP or to fragments of NTP and includes homologues, derivatives, variants, fusion proteins, and peptide mimetics of such peptides unless the context indicates otherwise. The expression "NTP peptide" also includes (but is not limited to) the peptides specifically listed in United States patent applications Serial Nos. 10/092,934, and 10/153334. The expression "NTP peptide" also preferably includes (but is not limited to) the following amino acid sequences of NTP:
- a) NTP peptide #1 [SEQ ID NO. 10], similar to AD7c-NTP p227-245 with the insertion of an additional phenylalanine residue after p228.
- PGFFKLFSCPSLLSSWDYRR
 Pro-Gly-Phe-Phe-Lys-Leu-Phe-Ser-Cys-Pro-Ser-Leu-Leu-Ser-Ser-Trp-Asp-Tyr-Arg-Arg
 corresponding nucleic acid sequence [SEQ ID NO. 10A]
 CCCGGGTTCTTCAAGTTATTCTCTGCCCCAGCCTCCTGAG
 TAGCTGGGACTACAGGCGC

- b) NTP peptide #2 [SEQ ID NO. 11], AD7c-NTP p114-132
 PELKQSTCLSLPKCWDYRR
 Pro-Glu-Leu-Lys-Gln-Ser-Thr-Cys-Leu-Ser-Leu-Pro-Lys-Cys-Trp-
 Asp-Tyr-Arg-Arg
 5 corresponding nucleic acid sequence [SEQ ID NO. 11A]
 CCTGAGCTCAAGCAGTCCACCTGCCTCAGCCTCCCAAAGT
 GCTGGGATTACAGGCGT
- c) NTP peptide #3 [SEQ ID NO. 12], AD7c-NTP p326-344
 10 PPGLKRFSCSLPSSWDYRG
 Pro-Pro-Gly-Leu-Lys-Arg-Phe-Ser-Cys-Leu-Ser-Leu-Pro-Ser-Ser-
 Trp-Asp-Tyr-Gly
 corresponding nucleic acid sequence [SEQ ID NO. 12A]
 CCTCCCGGGCTCAAGCGATTCTCCTGTCTCAGCCTCCCAA
 15 GCAGCTGGGATTACGGG
- d) NTP peptide #4 [SEQ ID NO. 13], AD7c-NTP p332-345
 FSCLSLPSSWDYGH
 Phe-Ser-Cys-Leu-Ser-Leu-Pro-Ser-Ser-Trp-Asp-Tyr-Gly-His
 20 corresponding nucleic acid sequence [SEQ ID NO. 13A]
 TTCTCCTGTCTCAGCC'CCCAAGCAGCTGGGATTACGGGC
 AC
- e) NTP peptide #5 [SEQ ID NO. 14], AD7c-NTP p119-132
 25 STCLSLPKCWDYRR
 Ser-Thr-Cys-Leu-Ser-Leu-Pro-Lys-Cys-Trp-Asp-Tyr-Arg-Arg
 corresponding nucleic acid sequence [SEQ ID NO. 14A]
 TCCACCTGCCTCAGCCTCCCAAAGTGCTGGGATTACAGGC
 30 GT
- f) NTP peptide #6 [SEQ ID NO. 15], AD7c-NTP p232-245

FSCPSLLSSWDYRR
 Phe-Ser-Cys-Pro-Ser-Leu-Leu-Ser-Ser-Trp-Asp-Tyr-Arg-Arg
 corresponding nucleic acid sequence [SEQ ID NO. 15A]
 TTCTCCTGCCCCAGCCTCCTGAGTAGCTGGGACTACAGGC
 5 GC

g) NTP peptide #7 [SEQ ID NO. 16], AD7c-NTP p335-343
 LSLPSSWDY
 Leu-Ser-Leu-Pro-Ser-Ser-Trp-Asp-Tyr-Gly
 10 corresponding nucleic acid sequence [SEQ ID NO. 16A]
 CTCAGCCTCCCAAGCAGCTGGGATTAC

h) NTP peptide #8 [SEQ ID NO. 17], AD7c-NTP p122-132
 LSLPKCWDYRR
 15 Leu-Ser-Leu-Pro-Lys-Cys-Trp-Asp-Tyr-Arg-Arg
 corresponding nucleic acid sequence [SEQ ID NO. 17A]
 CTCAGCCTCCCAAAGTGCTGGGATTACAGGCGT

i) NTP peptide #9 [SEQ ID NO. 18], AD7c-NTP p236-245
 20 SLLSSWDYRR
 Ser-Leu-Leu-Ser-Ser-Trp-Asp-Tyr-Arg-Arg
 corresponding nucleic acid sequence [SEQ ID NO. 18A]
 AGCCTCCTGAGTAGCTGGGACTACAGGCGC

25 j) NTP peptide #10 [SEQ ID NO. 19], AD7c-NTP p239-245 with the
 addition of leucine and proline residues at the N-terminal.
 LPSSWDYRR
 Leu-Pro-Ser-Ser-Trp-Asp-Tyr-Arg-Arg
 corresponding nucleic acid sequence [SEQ ID NO. 19A]
 30 CTCCCAGAGTAGCTGGGACTACAGGCGC

- k) NTP peptide #11 [SEQ ID NO. 20], AD7c-NTP p239-245
SSWDYRR
Ser-Ser-Trp-Asp-Tyr-Arg-Arg
corresponding nucleic acid sequence [SEQ ID NO. 20A]
5 GAGTAGCTGGGACTACAGGCGC
- l) NTP peptide #12 [SEQ ID NO. 21], AD7c-NTP p239-243, p339-343
SSWDY
Ser-Ser-Trp-Asp-Tyr
10 corresponding nucleic acid sequence [SEQ ID NO. 21A]
AGCAGCTGGGATTAC
- m) NTP peptide #13 [SEQ ID NO. 22], AD7c-NTP p239-245 + AD7c-NTP
p139-144
15 SSWDYRRFILEFL
Ser-Ser-Trp-Asp-Tyr-Arg-Arg-Phe-Ile-Leu-Phe-Phe-Leu
corresponding nucleic acid sequence [SEQ ID NO. 22A]
GAGTAGCTGGGACTACAGGCGCTTTATTTTATTTTTTTAA
- 20 n) NTP peptide #14 [SEQ ID NO. 23], AD7c-NTP p241-245 +AD7c-NTP
p197-202
WDYRRFIFNFL
Trp-Asp-Tyr-Arg-Arg-Phe-Ile-Phe-Asn-Phe-Leu
corresponding nucleic acid sequence [SEQ ID NO. 23A]
25 TGGGACTACAGGCGCTTTATTTTAAATTTTGTG
- o) NTP peptide #15 [SEQ ID NO. 24], AD7c-NTP p297-302
FNFCLF
Phe-Asn-Phe-Cys-Leu-Phe
30 corresponding nucleic acid sequence [SEQ ID NO. 24A]
TTTAATTTTGTGTTGTTT

- p) NTP peptide #16 [SEQ ID NO. 25], AD7c-NTP p197-202
 FIFNFL
 Phe-Ile-Phe-Asn-Phe-Leu
 5 corresponding nucleic acid sequence [SEQ ID NO. 25A]
 TTTATTTTAAATTTTGTG
- q) NTP peptide #17 [SEQ ID NO. 26], AD7c-NTP p31-43
 PASASPVAGITGM
 10 Pro-Ala-Ser-Ala-Ser-Pro-Val-Ala-Gly-Ile-Thr-Gly-Met
 corresponding nucleic acid sequence [SEQ ID NO. 26A]
 CCTGCCTCAGCCTCCCCAGTAGCTGGGATTACAGGCATG
- r) NTP peptide #18 [SEQ ID NO. 27], AD7c-NTP p175-187
 15 PASASQVAGTKDM
 Pro-Ala-Ser-Ala-Ser-Gln-Val-Ala-Gly-Thr-Lys-Asp-Met
 corresponding nucleic acid sequence [SEQ ID NO. 27A]
 CCTGCCTCAGCCTCCCAAGTAGCTGGGACCAAAGACATG
- s) NTP peptide #19 [SEQ ID NO. 28], AD7c-NTP p277-289
 20 PASASQSAGITGV
 Pro-Ala-Ser-Ala-Ser-Gln-Ser-Ala-Gly-Ile-Thr-Gly-Val
 corresponding nucleic acid sequence [SEQ ID NO. 28A]
 CCTGCCTCGGCCTCCCAAAGTGCTGGGATTACAGGCGTG
- 25 and includes homologues, derivatives, variants, fragments, fusion proteins, and
 peptide mimetics of these specifically listed NTP peptides and their corresponding
 nucleic acid sequences.
- The term "fragment" refers to a protein or polypeptide that consists of a
 30 continuous subsequence of the amino acid sequence of an NTP protein or NTP
 peptide and includes naturally occurring fragments such as splice variants and

fragments resulting from naturally occurring *in vivo* protease activity. Such a fragment may be truncated at the amino terminus, the carboxy terminus, and/or internally (such as by natural splicing). Such fragments may be prepared with or without an amino terminal methionine. The term “fragment” includes fragments, 5 whether identical or different, from the same NTP protein or NTP peptide, with a contiguous amino acid sequence in common or not, joined together, either directly or through a linker.

The term “variant” refers to a protein or polypeptide in which one or more amino acid substitutions, deletions, and/or insertions are present as compared to the 10 amino acid sequence of an NTP protein or NTP peptide and includes naturally occurring allelic variants or alternative splice variants of an NTP protein or NTP peptide. The term “variant” includes the replacement of one or more amino acids in a peptide sequence with a similar or homologous amino acid(s) or a dissimilar amino acid(s). There are many scales on which amino acids can be ranked as 15 similar or homologous. (Gunnar von Heijne, *Sequence Analysis in Molecular Biology*, p. 123-39 (Academic Press, New York, NY 1987.) Preferred variants include alanine substitutions at one or more of amino acid positions. Other preferred substitutions include conservative substitutions that have little or no effect on the overall net charge, polarity, or hydrophobicity of the protein. 20 Conservative substitutions are set forth in Table 2 below.

TABLE 2	
Conservative Amino Acid Substitutions	
5	Basic: arginine
	lysine
	histidine
	Acidic: glutamic acid
	aspartic acid
10	Uncharged Polar: glutamine
	asparagine
	serine
	threonine
	tyrosine
15	Non-Polar: phenylalanine
	tryptophan
	cysteine
	glycine
	alanine
20	valine
	proline
	methionine
	leucine
	isoleucine

Table 3 sets out another scheme of amino acid substitution:

TABLE 3

	Original Residue	Substitutions
5	Ala	gly; ser
	Arg	lys
	Asn	gln; his
	Asp	glu
	Cys	ser
10	Gln	asn
	Glu	asp
	Gly	ala; pro
	His	asn; gln
	Ile leu; val	
15	Leu	ile; val
	Lys	arg; gln; glu
	Met	leu; tyr; ile
	Phe	met; leu; tyr
	Ser	thr
20	Thr	ser
	Trp	tyr
	Tyr	trp; phe
	Val	ile; leu
25	Other variants can consist of less conservative amino acid substitutions, such as selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain.	
30	The substitutions that in general are expected to have a more significant effect on function are those in which (a) glycine and/or proline is substituted by another amino acid or is deleted or inserted; (b) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl, or alanyl; (c) a cysteine residue is substituted for (or by) any	
35	other residue; (d) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) a residue having an electronegative charge, e.g., glutamyl or aspartyl; or (e) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having such a side chain, e.g.,	

glycine. Other variants include those designed to either generate a novel glycosylation and/or phosphorylation site(s), or those designed to delete an existing glycosylation and/or phosphorylation site(s). Variants include at least one amino acid substitution at a glycosylation site, a proteolytic cleavage site and/or a cysteine residue. Variants also include NTP proteins and NTP peptides with additional amino acid residues before or after the NTP protein or NTP peptide amino acid sequence on linker peptides. For example, a cysteine residue may be added at both the amino and carboxy terminals of a in order to allow the cyclisation of the by the formation of a di-sulphide bond. The term “variant” also encompasses polypeptides that have the amino acid sequence of an NTP peptide with at least one and up to 25 or more additional amino acids flanking either the 3’ or 5’ end of the NTP peptide .

The term “derivative” refers to a chemically modified protein or polypeptide that has been chemically modified either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques, such as, for example, by addition of one or more polyethylene glycol molecules, sugars, phosphates, and/or other such molecules, where the molecule or molecules are not naturally attached to wild-type NTP protein or NTP peptide. Derivatives include salts. Such chemical modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art. It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given protein or polypeptide. Also, a given protein or polypeptide may contain many types of modifications. Modifications can occur anywhere in a protein or polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini. Modifications include, for example, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links,

- formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, glycosylation, lipid attachment, sulfation, gamma-
- 5 carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins, such as arginylation, and ubiquitination. See, for instance, *Proteins--Structure And Molecular Properties*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993) and Wold, F., "Posttranslational Protein
- 10 Modifications: Perspectives and Prospects," pgs. 1-12 in *Posttranslational Covalent Modification Of Proteins*, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., *Meth. Enzymol.* 182:626-646 (1990) and Rattan et al., "Protein Synthesis: Posttranslational Modifications and Aging," *Ann. N.Y. Acad. Sci.* 663: 48-62 (1992). The term "derivatives" include chemical modifications
- 15 resulting in the protein or polypeptide becoming branched or cyclic, with or without branching. Cyclic, branched and branched circular proteins or polypeptides may result from post-translational natural processes and may be made by entirely synthetic methods, as well.

- The term "homologue" refers to a protein that is at least 60 percent identical
- 20 in its amino acid sequence of an NTP protein or NTP peptide, as the case may be, as determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. The degree of similarity or identity between two proteins can be readily calculated by known methods, including but not limited to those described in *Computational Molecular*
- 25 *Biology*, Lesk, A. M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D. W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; *Sequence Analysis*
- 30 *Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo H. and Lipman, D., SIAM, *J. Applied Math.*, 48: 1073 (1988).

Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs.

- Preferred computer program methods useful in determining the identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., *et al.*, *Nucleic Acids Research*, 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA, Atschul, S. F. *et al.*, *J. Molec. Biol.*, 215: 403-410 (1990). The **BLAST** X program is publicly available from NCBI and other sources (**BLAST** Manual, Altschul, S., *et al.*, NCBI NLM NIH Bethesda, Md. 20894; Altschul, S., *et al.*, *J. Mol. Biol.*, 215: 403-410 (1990). By way of example, using a computer algorithm such as GAP (Genetic Computer Group, University of Wisconsin, Madison, Wis.), the two proteins or polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their respective amino acids (the "matched span", as determined by the algorithm).
- A gap opening penalty (which is calculated as 3 x (times) the average diagonal; the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid by the particular comparison matrix) and a gap extension penalty (which is usually 1/10 times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the algorithm. A standard comparison matrix (*see* Dayhoff *et al.* in: *Atlas of Protein Sequence and Structure*, vol. 5, supp.3 [1978] for the PAM250 comparison matrix; *see* Henikoff *et al.*, *Proc. Natl. Acad. Sci USA*, 89:10915-10919 [1992] for the BLOSUM 62 comparison matrix) also may be used by the algorithm. The percent identity then is calculated by the algorithm. Homologues will typically have one or more amino acid substitutions, deletions, and/or insertions as compared with the comparison NTP protein or NTP peptide, as the case may be.

- The term "peptide mimetic" or "mimetic" refers to biologically active compounds that mimic the biological activity of a peptide or a protein but are no longer peptidic in chemical nature, that is, they no longer contain any peptide bonds (that is, amide bonds between amino acids). Here, the term peptide mimetic

is used in a broader sense to include molecules that are no longer completely peptidic in nature, such as pseudo-peptides, semi-peptides and peptoids. Examples of peptide mimetics in this broader sense (where part of a peptide is replaced by a structure lacking peptide bonds) are described below. Whether completely or
5 partially non-peptide, peptide mimetics according to this invention provide a spatial arrangement of reactive chemical moieties that closely resemble the three-dimensional arrangement of active groups in the NTP peptide on which the peptide mimetic is based. As a result of this similar active-site geometry, the peptide mimetic has effects on biological systems that are similar to the biological activity
10 of the NTP peptide.

The peptide mimetics of this invention are preferably substantially similar in both three-dimensional shape and biological activity to the NTP peptides described herein. Examples of methods of structurally modifying a peptide known in the art to create a peptide mimetic include the inversion of backbone chiral
15 centers leading to D-amino acid residue structures that may, particularly at the N-terminus, lead to enhanced stability for proteolytical degradation without adversely affecting activity. An example is described in the paper "Tritiated D-ala¹-Peptide T Binding", Smith C. S. *et al.*, *Drug Development Res.*, 15, pp. 371-379 (1988). A second method is altering cyclic structure for stability, such as N to C interchain
20 imides and lactams (Ede *et al.* in Smith and Rivier (Eds.) "Peptides: Chemistry and Biology", Escom, Leiden (1991), pp. 268-270). An example of this is provided in conformationally restricted thymopentin-like compounds, such as those disclosed in U.S. Pat. No. 4,457,489 (1985), Goldstein, G. *et al.*, the disclosure of which is incorporated by reference herein in its entirety. A third method is to
25 substitute peptide bonds in the NTP peptide by pseudopeptide bonds that confer resistance to proteolysis.

A number of pseudopeptide bonds have been described that in general do not affect peptide structure and biological activity. One example of this approach is to substitute retro-inverso pseudopeptide bonds ("Biologically active
30 retroinverso analogues of thymopentin", Sisto A. *et al.* in Rivier, J. E. and Marshall, G. R. (eds) "Peptides, Chemistry, Structure and Biology", Escom, Leiden (1990),

pp. 722-773) and Dalpozzo, *et al.* (1993), *Int. J. Peptide Protein Res.*, 41:561-566, incorporated herein by reference). According to this modification, the amino acid sequences of the peptides may be identical to the sequences of an NTP peptide described above, except that one or more of the peptide bonds are replaced by a
5 retro-inverso pseudopeptide bond. Preferably the most N-terminal peptide bond is substituted, since such a substitution will confer resistance to proteolysis by exopeptidases acting on the N-terminus. Further modifications also can be made by replacing chemical groups of the amino acids with other chemical groups of similar structure. Another suitable pseudopeptide bond that is known to enhance
10 stability to enzymatic cleavage with no or little loss of biological activity is the reduced isostere pseudopeptide bond (Couder, *et al.* (1993), *Int. J. Peptide Protein Res.*, 41:181-184, incorporated herein by reference in its entirety).

Thus, the amino acid sequences of these peptides may be identical to the sequences of an NTP peptide, except that one or more of the peptide bonds are
15 replaced by an isostere pseudopeptide bond. The expression "amino acid sequence(s)" preferably is used herein to denote a sequence of at least two amino acids, preferably at least four, and more preferably at least five. Preferably the most N-terminal peptide bond is substituted, since such a substitution would confer resistance to proteolysis by exopeptidases acting on the N-terminus. The synthesis
20 of peptides with one or more reduced isostere pseudopeptide bonds is known in the art (Couder, *et al.* (1993), cited above). Other examples include the introduction of ketomethylene or methylsulfide bonds to replace peptide bonds.

Peptoid derivatives of NTP peptides represent another class of peptide mimetics that retain the important structural determinants for biological activity,
25 yet eliminate the peptide bonds, thereby conferring resistance to proteolysis (Simon, *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, 89:9367-9371, incorporated herein by reference in its entirety). Peptoids are oligomers of N-substituted glycines. A number of N-alkyl groups have been described, each corresponding to the side chain of a natural amino acid (Simon, *et al.* (1992), cited above). Some or
30 all of the amino acids of the NTP peptides may be replaced with the N-substituted glycine corresponding to the replaced amino acid.

The expression “peptide mimetic” or “mimetic” also includes reverse-D peptides and enantiomers as defined below. The phrase “reverse-D peptide” refers to a biologically active protein or peptide consisting of D-amino acids arranged in a reverse order as compared to the L-amino acid sequence of an NTP peptide. Thus,
5 the carboxy terminal residue of an L-amino acid NTP peptide becomes the amino terminal for the D-amino acid peptide and so forth. For example, the NTP peptide, SSWDY, becomes Y_dD_dW_dS_dS_d, where D_d, S_d, W_d, and Y_d are the D-amino acids corresponding to the L-amino acids, D, S, W, and Y respectively.

“Enantiomer” as it is used herein, refers to a biologically active protein or
10 peptide where one or more the L-amino acid residues in the amino acid sequence of an NTP peptide is replaced with the corresponding D-amino acid residue(s).

Amino acids and amino acid residues described herein may be referred to according to the accepted one or three-letter code provided in the table below.

Table 1

Amino Acid	One-Letter Symbol	Three-Letter Symbol
Alanine	A	Ala
5 Arginine	R	Arg
Asparagine	N	Asn
Aspartic acid	D	Asp
Cysteine	C	Cys
Glutamine	Q	Gln
10 Glutamic acid	E	Glu
Glycine	G	Gly
Histidine	H	His
Isoleucine	I	Ile
Leucine	L	Leu
15 Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
20 Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val

25 The present invention is directed to a composition comprising NTP peptides as defined above in this invention. A preferred NTP peptide is similar to or homologous to an NTP peptide derived from AD7c-NTP. However, the use of other NTP peptides based on portions or fragments of other species of NTP proteins also is encompassed by the invention. For example, the AD7c-NTP

30 peptide sequences and similar variants and homologs also are found in a wide variety of human and non-human proteins ("Related Proteins"). In particular, the AD7c-NTP gene contains Alu-type sequences that are closely similar to those also found in other genes in the human and other primate genomes.

It therefore is reasonable to expect that some, if not all, of the Related

35 Proteins also will prove to be effective agents for causing cell death because they contain peptide sequences homologous or closely similar to the AD7c-NTP peptides ("Related Peptides"). Similarly a person ordinarily skilled in the art could

synthesize specific Related Peptides based on the amino acid sequence for any Related Protein found to be an effective agent for causing cell death and test them for efficacy as agents for causing cell death.

Other peptide sequences derived from a Related Protein found to be an effective agent for causing cell death also may be effective agents for causing cell death. A person ordinarily skilled in the art can synthesize without undue experimentation fragments of an effective Related Protein spanning the entire amino acid sequence of that protein in order to identify other effective peptide sequences.

The preferred NTP peptides of the present invention include proteins and peptides known to contain amino acid sequences identical, closely similar to or homologous to NTP peptide sequences.

NTP peptides and fragments, variants, derivatives, homologues and mimetics thereof encompassed by this invention can be prepared using methods known to those of skilled in the art, such as recombinant DNA technology, protein synthesis and isolation of naturally occurring NTP peptides and fragments, variants, derivatives and homologues thereof.

An NTP peptide can be prepared using well known recombinant DNA technology methods such as those set forth in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. [1989]) and/or Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, Green Publishers Inc. and Wiley and Sons, N.Y. [1994].

A gene or cDNA encoding an NTP peptide may be obtained for example by screening a genomic or cDNA library, or by PCR amplification. Probes or primers useful for screening the library can be generated based on sequence information for other known genes or gene fragments from the same or a related family of genes, such as, for example, conserved motifs found in other NTP peptides or NTP proteins. In addition, where a gene encoding an NTP peptide or NTP protein has been identified from one species, all or a portion of that gene may be used as a probe to identify homologous genes from other species. The probes or primers may be used to screen cDNA libraries from various tissue sources believed to

express an NTP peptide or NTP protein gene. Typically, conditions of high stringency will be employed for screening to minimize the number of false positives obtained from the screen.

Another means to prepare a gene encoding an NTP peptide or NTP protein is to employ chemical synthesis using methods well known to the skilled artisan, such as those described by Engels *et al.* (Angew. Chem. Intl. Ed., 28:716-734 [1989]). These methods include, *inter alia*, the phosphotriester, phosphoramidite, and H-phosphonate methods for nucleic acid synthesis. A preferred method for such chemical synthesis is polymer-supported synthesis using standard phosphoramidite chemistry. Typically, the DNA encoding an NTP peptide or NTP protein will be several hundred nucleotides in length. Nucleic acids larger than about 100 nucleotides can be synthesized as several fragments using these methods. The fragments then can be ligated together to form the full length NTP peptide or NTP protein. Usually, the DNA fragment encoding the amino terminus of the protein will have an ATG, which encodes a methionine residue. This methionine may or may not be present on the mature form of the NTP protein or NTP peptide, depending on whether the protein produced in the host cell is designed to be secreted from that cell.

The gene, cDNA, or fragment thereof encoding the NTP protein or NTP peptide can be inserted into an appropriate expression or amplification vector using standard ligation techniques. The vector typically is selected to be functional in the particular host cell employed (i.e., the vector is compatible with the host cell machinery such that amplification of the gene and/or expression of the gene can occur). The gene, cDNA or fragment thereof encoding the NTP protein or NTP peptide may be amplified/expressed in prokaryotic, yeast, insect (*baculovirus* systems) and/or eukaryotic host cells. Selection of the host cell will depend in part on whether the NTP protein or NTP peptide is to be glycosylated and/or phosphorylated. If so, yeast, insect, or mammalian host cells are preferable.

Typically, the vectors used in any of the host cells will contain 5' flanking sequence (also referred to as a promoter) and other regulatory elements, such as an enhancer(s), an origin of replication element, a transcriptional termination element,

a complete intron sequence containing a donor and acceptor splice site, a signal peptide sequence, a ribosome binding site element, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Each of these elements is discussed
5 below. Optionally, the vector may contain a tag sequence, i.e., an oligonucleotide molecule located at the 5' or 3' end of the NTP protein or NTP peptide coding sequence; the oligonucleotide molecule encodes polyHis (such as hexaHis), or other tag such as FLAG, HA (hemagglutinin Influenza virus) or myc for which commercially available antibodies exist. This tag typically is fused to the
10 polypeptide upon expression of the polypeptide, and can serve as a means for affinity purification of the NTP protein or NTP peptide from the host cell. Affinity purification can be accomplished, for example, by column chromatography using antibodies against the tag as an affinity matrix. Optionally, the tag can subsequently be removed from the purified NTP protein or NTP peptide by various
15 means such as using certain peptidases.

The human immunoglobulin hinge and Fc region could be fused at either the N-terminus or C-terminus of the NTP protein or NTP peptide by one skilled in the art. The subsequent Fc-fusion protein could be purified by use of a Protein A affinity column. Fc is known to exhibit a long pharmacokinetic half-life *in vivo*
20 and proteins fused to Fc have been found to exhibit a substantially greater half-life *in vivo* than the unfused counterpart. Also, fusion to the Fc region allows for dimerization/multimerization of the molecule that may be useful for the bioactivity of some molecules.

The 5' flanking sequence may be homologous (i.e., from the same species
25 and/or strain as the host cell), heterologous (i.e., from a species other than the host cell species or strain), hybrid (i.e., a combination of 5' flanking sequences from more than one source), synthetic, or it may be the native NTP protein or NTP peptide gene 5' flanking sequence. As such, the source of the 5' flanking sequence may be any unicellular prokaryotic or eukaryotic organism, any vertebrate or
30 invertebrate organism, or any plant, provided that the 5' flanking sequence is functional in, and can be activated by, the host cell machinery.

The 5' flanking sequences useful in the vectors of this invention may be obtained by any of several methods well known in the art. Typically, 5' flanking sequences useful herein other than the NTP protein or NTP peptide gene flanking sequence will have been previously identified by mapping and/or by restriction
5 endonuclease digestion and can thus be isolated from the proper tissue source using the appropriate restriction endonucleases. In some cases, the full nucleotide sequence of the 5' flanking sequence may be known. Here, the 5' flanking sequence may be synthesized using the methods described above for nucleic acid synthesis or cloning.

10 Where all or only a portion of the 5' flanking sequence is known, it may be obtained using PCR and/or by screening a genomic library with suitable oligonucleotide and/or 5' flanking sequence fragments from the same or another species.

Where the 5' flanking sequence is not known, a fragment of DNA
15 containing a 5' flanking sequence may be isolated from a larger piece of DNA that may contain, for example, a coding sequence or even another gene or genes. Isolation may be accomplished by restriction endonuclease digestion using one or more carefully selected enzymes to isolate the proper DNA fragment. After digestion, the desired fragment may be isolated by agarose gel purification,
20 Qiagen® column or other methods known to the skilled artisan. Selection of suitable enzymes to accomplish this purpose will be readily apparent to one of ordinary skill in the art.

The origin of replication element is typically a part of prokaryotic expression vectors purchased commercially, and aids in the amplification of the
25 vector in a host cell. Amplification of the vector to a certain copy number can, in some cases, be important for optimal expression of the NTP protein or NTP peptide. If the vector of choice does not contain an origin of replication site, one may be chemically synthesized based on a known sequence, and ligated into the vector. The transcription termination element is typically located 3' of the end of
30 the NTP protein or NTP peptide coding sequence and serves to terminate transcription of the NTP protein or NTP peptide. Usually, the transcription

termination element in prokaryotic cells is a G-C rich fragment followed by a poly T sequence. While the element may be cloned from a library or purchased commercially as part of a vector, it also can be readily synthesized using methods for nucleic acid synthesis such as those described above.

5 A selectable marker gene element encodes a protein necessary for the survival and growth of a host cell grown in a selective culture medium. Typical selection marker genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, tetracycline, or kanamycin for prokaryotic host cells, (b) complement auxotrophic deficiencies of the cell; or (c) supply critical nutrients
10 not available from complex media. Preferred selectable markers are the kanamycin resistance gene, the ampicillin resistance gene, and the tetracycline resistance gene.

The ribosome binding element, commonly called the Shine-Dalgarno sequence (prokaryotes) or the Kozak sequence (eukaryotes), is usually necessary for translation initiation of mRNA. The element is typically located 3' to the
15 promoter and 5' to the coding sequence of the NTP protein or NTP peptide to be synthesized. The Shine-Dalgarno sequence is varied but is typically a polypurine (i.e., having a high A-G content). Many Shine-Dalgarno sequences have been identified, each of which can be readily synthesized by a person skilled in the art using methods set forth above and used in a prokaryotic vector.

20 In those cases where it is desirable for NTP protein or NTP peptide to be secreted from the host cell, a signal sequence may be used to direct the NTP protein or NTP peptide out of the host cell where it is synthesized, and the carboxy-terminal part of the protein may be deleted in order to prevent membrane anchoring. Typically, the signal sequence is positioned in the coding region of the
25 NTP protein /NTP peptide gene or cDNA, or directly at the 5' end of the NTP protein/NTP peptide gene coding region. Many signal sequences have been identified, and any of them that are functional in the selected host cell may be used in conjunction with the NTP protein/NTP peptide gene or cDNA. Therefore, the signal sequence may be homologous or heterologous to the NTP protein/NTP
30 peptide gene or cDNA, and may be homologous or heterologous to the NTP protein/NTP peptide gene or cDNA. Additionally, the signal sequence may be

chemically synthesized using methods set forth above. In most cases, secretion of the polypeptide from the host cell via the presence of a signal peptide will result in the removal of the amino terminal methionine from the polypeptide.

In many cases, transcription of the NTP protein/NTP peptide gene or cDNA is increased by the presence of one or more introns in the vector; this is particularly true where the NTP protein or NTP peptide is produced in eukaryotic host cells, especially mammalian host cells. The introns used may be naturally occurring within the NTP protein/NTP peptide gene, especially where the gene used is a full length genomic sequence or a fragment thereof. Where the intron is not naturally occurring within the gene (as for most cDNAs), the intron(s) may be obtained from another source. The position of the intron with respect to the flanking sequence and the NTP protein/NTP peptide gene is generally important, as the intron must be transcribed to be effective. As such, where the NTP protein/NTP peptide gene is inserted into the expression vector is a cDNA molecule, the preferred position for the intron is 3' to the transcription start site, and 5' to the polyA transcription termination sequence. Preferably for NTP protein/NTP peptide cDNA, the intron or introns will be located on one side or the other (i.e., 5' or 3') of the cDNA such that it does not interrupt this coding sequence. Any intron from any source, including any viral, prokaryotic and eukaryotic (plant or animal) organisms, may be used to practice this invention, provided that it is compatible with the host cell(s) into which it is inserted. Also included herein are synthetic introns. Optionally, more than one intron may be used in the vector.

Where one or more of the elements set forth above are not already present in the vector to be used, they may be individually obtained and ligated into the vector. Methods used for obtaining each of the elements are well known to the skilled artisan and are comparable to the methods set forth above (i.e., synthesis of the DNA, library screening, and the like).

The final vectors used to practice this invention typically are constructed from starting vectors such as a commercially available vector. Such vectors may or may not contain some of the elements to be included in the completed vector. If none of the desired elements are present in the starting vector, each element may be

individually ligated into the vector by cutting the vector with the appropriate restriction endonuclease(s) such that the ends of the element to be ligated in and the ends of the vector are compatible for ligation. In some cases, it may be necessary to blunt the ends to be ligated together in order to obtain a satisfactory ligation.

- 5 Blunting is accomplished by first filling in "sticky ends" using Klenow DNA polymerase or T4 DNA polymerase in the presence of all four nucleotides. This procedure is well known in the art and is described for example in Sambrook *et al.*, *supra*. Alternatively, two or more of the elements to be inserted into the vector may first be ligated together (if they are to be positioned adjacent to each other)
- 10 and then ligated into the vector.

- An additional method for constructing the vector is to conduct all ligations of the various elements simultaneously in one reaction mixture. Here, many nonsense or nonfunctional vectors will be generated due to improper ligation or insertion of the elements. The functional vector may be identified and selected,
- 15 however, by restriction endonuclease digestion.

- Preferred vectors for practicing this invention are those that are compatible with bacterial, insect, and mammalian host cells. Such vectors include, inter alia, pCRII, pCR3, and pcDNA3.1 (Invitrogen Company, San Diego, Calif.), pBSII (Stratagene Company, La Jolla, Calif.), pET15b (Novagen, Madison, Wis.), PGEX
- 20 (Pharmacia Biotech, Piscataway, N.J.), pEGFP-N2 (Clontech, Palo Alto, Calif.), pETL (BlueBacII; Invitrogen), and pFastBacDual (Gibco/BRL, Grand Island, N.Y.).

- After the vector has been constructed and a nucleic acid molecule encoding full length or truncated NTP protein or NTP peptide has been inserted into the
- 25 proper site of the vector, the completed vector may be inserted into a suitable host cell for amplification and/or polypeptide expression. Host cells may be prokaryotic host cells (such as *E. coli*) or eukaryotic host cells (such as a yeast cell, an insect cell, or a vertebrate cell). The host cell, when cultured under appropriate conditions, can synthesize NTP protein or NTP peptide which can subsequently be
- 30 collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted).

After collection, the NTP protein or NTP peptide can be purified using methods such as molecular sieve chromatography, affinity chromatography, and the like. Selection of the host cell for NTP protein or NTP peptide production will depend in part on whether the NTP protein or NTP peptide is to be glycosylated or phosphorylated (in which case eukaryotic host cells are preferred), and the manner in which the host cell is able to fold the protein into its native tertiary structure (e.g., proper orientation of disulfide bridges, etc.) such that the biologically active protein is prepared by the NTP protein or NTP peptide that has biological activity. The NTP protein or NTP peptide may be folded after synthesis using appropriate chemical conditions as discussed below. Suitable cells or cell lines useful in the invention include mammalian cells, such as Chinese hamster ovary cells (CHO), human embryonic kidney (HEK) 293 or 293T cells, or 3T3 cells. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification can be accomplished by those skilled in the art using the guidelines provided herein. Other suitable mammalian cell lines, include the monkey COS-1 and COS-7 cell lines, and the CV-1 cell line. Further exemplary mammalian host cells include primate cell lines and rodent cell lines, including transformed cell lines. Normal diploid cells, cell strains derived from in vitro culture of primary tissue, as well as primary explants, are also suitable. Candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Other suitable mammalian cell lines include but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines.

Similarly useful as host cells suitable for the present invention are bacterial cells. For example, the various strains of *E. coli* (e.g., HB101, DH5.alpha., DH10, and MC1061) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas spp.*, other *Bacillus spp.*, *Streptomyces spp.*, and the like may also be employed in this method. Many strains of yeast cells known to those skilled in the art also are available as host cells for expression of the polypeptides of the present invention.

Additionally, where desired, insect cell systems may be utilized in the methods of the present invention. Such systems are described for example in Kitts et al. (*Biotechniques*, 14:810-817 [1993]), Lucklow (*Curr. Opin. Biotechnol.*, 4:564-572 [1993]) and Lucklow et al. (*J. Virol.*, 67:4566-4579 [1993]). Preferred
5 insect cells are Sf-9 and Hi5 (Invitrogen, Carlsbad, Calif.).

Insertion (also referred to as transformation or transfection) of the vector into the selected host cell may be accomplished using such methods as calcium chloride, electroporation, microinjection, lipofection, or the DEAE-dextran method. The method selected will in part be a function of the type of host cell to
10 be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook *et al.*, *supra*.

The host cells containing the vector (i.e., transformed or transfected) may be cultured using standard media well known to the skilled artisan. The media will usually contain all nutrients necessary for the growth and survival of the cells.
15 Suitable media for culturing *E. coli* cells are for example, Luria Broth (LB) and/or Terrific Broth (TB). Suitable media for culturing eukaryotic cells are RPMI 1640, MEM, DMEM, all of which may be supplemented with serum and/or growth factors as required by the particular cell line being cultured. A suitable medium for insect cultures is Grace's medium supplemented with yeastolate, lactalbumin
20 hydrolysate, and/or fetal calf serum as necessary. Typically, an antibiotic or other compound useful for selective growth of the transformed cells only is added as a supplement to the media. The compound to be used will be dictated by the selectable marker element present on the plasmid with which the host cell was transformed. For example, where the selectable marker element is kanamycin
25 resistance, the compound added to the culture medium will be kanamycin.

The amount of NTP protein or NTP peptide produced in the host cell can be evaluated using standard methods known in the art. Such methods include, without limitation, Western blot analysis, SDS-polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis, HPLC separation, mass spectroscopy,
30 immunoprecipitation, and/or activity assays such as DNA binding gel shift assays.

If the NTP protein or NTP peptide has been designed to be secreted from the host cells, the majority of the NTP protein or NTP peptide may be found in the cell culture medium. Proteins prepared in this way will typically not possess an amino terminal methionine, as it is removed during secretion from the cell. If the
5 NTP protein or NTP peptide is not secreted from the host cells, however, it will be present in the cytoplasm and/or the nucleus (for eukaryotic host cells) or in the cytosol, (for gram negative bacteria host cells), and it may have an amino terminal methionine.

For NTP protein or NTP peptide situated in the host cell cytoplasm and/or
10 nucleus, the host cells typically are first disrupted mechanically or with detergent to release the intra-cellular contents into a buffered solution. NTP protein or NTP peptide then can be isolated from this solution.

Purification of NTP protein or NTP peptide from solution can be accomplished using a variety of techniques. If the protein has been synthesized
15 such that it contains a tag such as hexaHistidine (e.g. NTP peptide/hexaHis) or other small peptide such as FLAG (Sigma-Aldrich, St. Louis, MI) or calmodulin-binding peptide (Stratagene, La Jolla, CA) at either its carboxyl or amino terminus, it may essentially be purified in a one-step process by passing the solution through an affinity column where the column matrix has a high affinity for the tag or for the
20 protein directly (i.e., a monoclonal antibody specifically recognizing the NTP peptide). For example, polyhistidine binds with great affinity and specificity to nickel, zinc and cobalt; thus immobilized metal ion affinity chromatography which employs a nickel-based affinity resin (as used in Qiagen's QIAexpress system or Invitrogen's Xpress System) or a cobalt-based affinity resin (as used in BD
25 Biosciences-CLONTECH's Talon system) can be used for purification of NTP peptide/polyHis. (See for example, Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, Section 10.11.8, John Wiley & Sons, New York [1993]).

Where the NTP protein or NTP peptide is prepared without a tag attached, and no antibodies are available, other well known procedures for purification can
30 be used. Such procedures include, without limitation, ion exchange chromatography, hydroxyapatite chromatography, hydrophobic interaction

chromatography, molecular sieve chromatography, HPLC, native gel electrophoresis in combination with gel elution, and preparative isoelectric focusing (Isoprime machine/technique, Hoefer Scientific). In some cases, two or more of these techniques may be combined to achieve increased purity.

5 If it is anticipated that the NTP protein or NTP peptide will be found primarily intracellularly, the intracellular material (including inclusion bodies for gram-negative bacteria) can be extracted from the host cell using any standard technique known to the skilled artisan. For example, the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press, homogenization, 10 and/or sonication followed by centrifugation. If the NTP protein or NTP peptide has formed inclusion bodies in the cytosol, the inclusion bodies can often bind to the inner and/or outer cellular membranes and thus will be found primarily in the pellet material after centrifugation. The pellet material then can be treated at pH extremes or with chaotropic agent such as a detergent, guanidine, guanidine 15 derivatives, urea, or urea derivatives in the presence of a reducing agent such as dithiothreitol at alkaline pH or tris carboxyethyl phosphine at acid pH to release, break apart, and solubilize the inclusion bodies. The NTP protein or NTP peptide in its now soluble form then can be analyzed using gel electrophoresis, immunoprecipitation or the like. If it is desired to isolate the NTP protein or NTP 20 peptide, isolation may be accomplished using standard methods such as those set forth below and in Marston et al. (*Meth. Enz.*, 182:264-275 [1990]).

 In some cases, the NTP protein or NTP peptide may not be biologically active upon isolation. Various methods for refolding or converting the polypeptide to its tertiary structure and generating disulfide linkages, can be used to restore 25 biological activity. Such methods include exposing the solubilized polypeptide to a pH usually above 7 and in the presence of a particular concentration of a chaotrope. The selection of chaotrope is very similar to the choices used for inclusion body solubilization but usually at a lower concentration and is not necessarily the same chaotrope as used for the solubilization. In most cases the refolding/oxidation 30 solution also will contain a reducing agent or the reducing agent plus its, oxidized form in a specific ratio to generate a particular redox potential allowing for

disulfide shuffling to occur in the formation of the protein's cysteine bridge(s).

Some of the commonly used redox couples include cysteine/cystamine, glutathione (GSH)/dithiobis GSH, cupric chloride, dithiothreitol (DTT)/dithiane DTT, 2-mercaptoethanol(bME)/dithio-b(ME). In many instances a cosolvent may be
5 necessary to increase the efficiency of the refolding and the more common reagents used for this purpose include glycerol, polyethylene glycol of various molecular weights, and arginine.

If NTP protein or NTP peptide inclusion bodies are not formed to a significant degree in the host cell, the NTP protein or NTP peptide may be found
10 primarily in the supernatant after centrifugation of the cell homogenate, and the NTP protein or NTP peptide can be isolated from the supernatant using methods such as those set forth below.

In those situations where it is preferable to partially or completely isolate the NTP protein or NTP peptide, purification can be accomplished using standard
15 methods well known to the skilled artisan. Such methods include, without limitation, separation by electrophoresis followed by electroelution, various types of chromatography (immunoaffinity, molecular sieve, and/or ion exchange), and/or high pressure liquid chromatography. In some cases, it may be preferable to use more than one of these methods for complete purification.

In addition to preparing and purifying NTP proteins or NTP peptides using recombinant DNA techniques, the NTP proteins or NTP peptides and their fragments, variants, homologues and derivatives may be prepared by chemical synthesis methods (such as solid phase peptide synthesis) using techniques known
20 in the art such as those set forth by Merrifield *et al.*, (*J. Am. Chem. Soc.*, 85:2149 [1963]), Houghten *et al.* (*Proc Natl Acad. Sci. USA*, 82:5132 [1985]), and Stewart and Young (*Solid Phase Peptide Synthesis*, Pierce Chemical Co., Rockford, Ill. [1984]). Such polypeptides may be synthesized with or without a methionine on the amino terminus. Chemically synthesized NTP proteins or NTP peptides may be oxidized using methods set forth in these references to form disulfide bridges.
25
30 The NTP proteins or NTP peptides are expected to have biological activity comparable to NTP proteins or NTP peptides produced recombinantly or purified

from natural sources, and thus may be used interchangeably with recombinant or natural NTP protein or NTP peptide.

Chemically modified NTP protein or NTP peptide compositions in which the NTP protein or NTP peptide is linked to a polymer are included within the scope of the present invention. The polymer selected is typically water soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. The polymer selected is usually modified to have a single reactive group, such as an active ester for acylation or an aldehyde for alkylation, so that the degree of polymerization may be controlled as provided for in the present methods. The polymer may be of any molecular weight, and may be branched or unbranched. Included within the scope of //NTP peptide polymers is a mixture of polymers.

In some cases, it may be desirable to prepare nucleic acid and/or amino acid variants of the naturally occurring NTP proteins or NTP peptides. Nucleic acid variants may be produced using site directed mutagenesis, PCR amplification, or other appropriate methods, where the primer(s) have the desired point mutations (see Sambrook *et al.*, *supra*, and Ausubel *et al.*, *supra*, for descriptions of mutagenesis techniques). Chemical synthesis using methods described by Engels *et al.*, *supra*, may also be used to prepare such variants. Other methods known to the skilled artisan may be used as well.

Preferred nucleic acid variants are those containing nucleotide substitutions accounting for codon preference in the host cell that is to be used to produce the NTP protein or NTP peptide. Such codon optimization can be determined via computer algorithms which incorporate codon frequency tables such as Ecohigh. Cod for codon preference of highly expressed bacterial genes as provided by the University of Wisconsin Package Version 9.0, Genetics Computer Group, Madison, Wis. Other useful codon frequency tables include *Celegans_high.cod*, *Celegans_low.cod*, *Drosophila_high.cod*, *Human_high.cod*, *Maize_high.cod*, and *Yeast_high.cod*. Other preferred variants are those encoding conservative amino acid changes as described above (e.g., wherein the charge or polarity of the naturally occurring amino acid side chain is not altered substantially by substitution

with a different amino acid) as compared to wild type, and/or those designed to either generate a novel glycosylation and/or phosphorylation site(s), or those designed to delete an existing glycosylation and/or phosphorylation site(s).

NTP proteins, NTP peptides, and fragments, homologs, variants, derivatives
5 and salts thereof can be made using conventional peptide synthesis techniques known to one of ordinary skill in the art. These techniques include chemical coupling methods (cf. Wunsch, E: "Methoden der organischen Chemie", Volume 15, Band 1+2, *Synthese von Peptiden*, thime Verlag, Stuttgart (1974), and Barrany, G.; Marrifield, R. B.: "The Peptides", eds. E. Gross, J. Meienhofer, Volume 2,
10 Chapter 1, pp. 1-284, Academic Press (1980)), enzymatic coupling methods (cf. Widmer, F. Johansen, J. T., *Carlsberg Res. Commun.*, Vol. 44, pp. 37-46 (1979), and Kullmann, W.: "Enzymatic Peptide Synthesis", CRC Press Inc. Boca Raton, Fla. (1987), and Widmer, F., Johansen, J. T. in "Synthetic Peptides in Biology and Medicines", eds. Alitalo, K., Partanen, P., Väterli, A., pp.79-86, Elsevier,
15 Amsterdam (1985)), or a combination of chemical and enzymatic methods if this is advantageous for the process design and economy. Using the teachings provided in this disclosure, those skilled in the art are capable of varying the peptide sequence of the NTP peptide to make a homologue having the same or similar biological activity (bioactivity) as the original or native NTP protein or NTP peptide.

20 There can be advantages for using a mimetic of a given NTP protein or NTP peptide rather than the protein itself. In general, peptide mimetics are more bioavailable, have a longer duration of action and can be cheaper to produce than proteins and peptides.

Thus the NTP proteins and NTP peptides described above have utility in the
25 development of such small chemical compounds with similar biological activities and therefore with similar therapeutic utilities. Peptide mimetics of NTP proteins and NTP peptides can be developed using combinatorial chemistry techniques and other techniques known in the art (see e.g. Proceedings of the 20th European Peptide Symposium, ed. G. Jung, E. Bayer, pp. 289-336, and references therein).

30 Examples of methods of structurally modifying a peptide known in the art to create a peptide mimetic include the inversion of backbone chiral centers leading

to D-amino acid residue structures that may, particularly at the N-terminus, lead to enhanced stability for proteolytical degradation without adversely affecting activity. An example is described in the paper "Tritiated D-ala¹-Peptide T Binding", Smith C. S. *et al.*, *Drug Development Res.* 15, pp. 371-379 (1988).

5 A second method is altering cyclic structure for stability, such as N to C interchain imides and lactames (Ede *et al.* in Smith and Rivier (Eds.) "Peptides: Chemistry and Biology", Escom, Leiden (1991), pp. 268-270). An example of this is provided in conformationally restricted thymopentin-like compounds, such as those disclosed in U.S. Pat. No. 4,457,489 (1985), Goldstein, G. *et al.*, the
10 disclosure of which is incorporated by reference herein in its entirety.

 A third method is to substitute peptide bonds in the NTP protein or NTP peptide by pseudopeptide bonds that confer resistance to proteolysis. A number of pseudopeptide bonds have been described that in general do not affect peptide structure and biological activity. One example of this approach is to substitute
15 retro-inverso pseudopeptide bonds ("Biologically active retroinverso analogues of thymopentin", Sisto A. *et al.* in Rivier, J. E. and Marshall, G. R. (eds) "Peptides, Chemistry, Structure and Biology", Escom, Leiden (1990), pp. 722-773) and Dalpozzo, *et al.* (1993), *Int. J. Peptide Protein Res.*, 41:561-566, incorporated herein by reference). According to this modification, the amino acid sequences of
20 the peptides may be identical to the sequences of the NTP proteins and NTP peptides described above, except that one or more of the peptide bonds are replaced by a retro-inverso pseudopeptide bond. Preferably the most N-terminal peptide bond is substituted, since such a substitution will confer resistance to proteolysis by exopeptidases acting on the N-terminus.

25 The synthesis of peptides with one or more reduced retro-inverso pseudopeptide bonds is known in the art (Sisto (1990) and Dalpozzo, *et al.* (1993), cited above). Thus, peptide bonds can be replaced by non-peptide bonds that allow the peptide mimetic to adopt a similar structure, and therefore biological activity, to the original peptide. Further modifications also can be made by replacing chemical
30 groups of the amino acids with other chemical groups of similar structure. Another suitable pseudopeptide bond that is known to enhance stability to enzymatic

cleavage with no or little loss of biological activity is the reduced isostere pseudopeptide bond (Couder, *et al.* (1993), *Int. J. Peptide Protein Res.*, 41:181-184, incorporated herein by reference in its entirety). Thus, the amino acid sequences of these peptides may be identical to the sequences of an NTP protein or

5 NTP peptide, except that one or more of the peptide bonds are replaced by an isostere pseudopeptide bond. Preferably the most N-terminal peptide bond is substituted, since such a substitution would confer resistance to proteolysis by exopeptidases acting on the N-terminus. The synthesis of peptides with one or more reduced isostere pseudopeptide bonds is known in the art (Couder, *et al.*

10 (1993), cited above). Other examples include the introduction of ketomethylene or methylsulfide bonds to replace peptide bonds.

Peptoid derivatives of NTP proteins and NTP peptides represent another class of peptide mimetics that retain the important structural determinants for biological activity, yet eliminate the peptide bonds, thereby conferring resistance to

15 proteolysis (Simon, *et al.*, 1992, *Proc. Natl. Acad. Sci. USA*, 89:9367-9371 and incorporated herein by reference in its entirety). Peptoids are oligomers of N-substituted glycines. A number of N-alkyl groups have been described, each corresponding to the side chain of a natural amino acid (Simon, *et al.* (1992), cited above and incorporated herein by reference in its entirety). Some or all of the

20 amino acids of the NTP protein or NTP peptide are replaced with the N-substituted glycine corresponding to the replaced amino acid.

The development of peptide mimetics can be aided by determining the tertiary structure of the original NTP protein or NTP peptide by NMR spectroscopy, crystallography and/or computer-aided molecular modeling. These

25 techniques aid in the development of novel compositions of higher potency and/or greater bioavailability and/or greater stability than the original peptide (Dean (1994), *BioEssays*, 16: 683-687; Cohen and Shatzmiller (1993), *J. Mol. Graph.*, 11: 166-173; Wiley and Rich (1993), *Med. Res. Rev.*, 13: 327-384; Moore (1994), *Trends Pharmacol. Sci.*, 15: 124-129; Hruby (1993), *Biopolymers*, 33: 1073-1082;

30 Bugg *et al.* (1993), *Sci. Am.*, 269: 92-98, all incorporated herein by reference in their entirety).

Once a potential peptide mimetic compound is identified, it may be synthesized and assayed using the methods outlined in the examples below to assess its activity. The peptide mimetic compounds obtained by the above methods, having the biological activity of the NTP proteins or NTP peptides and similar three-dimensional structure, are encompassed by this invention. It will be readily apparent to one skilled in the art that a peptide mimetic can be generated from any of the NTP proteins or NTP Peptides bearing one or more of the modifications described above. It will furthermore be apparent that the peptide mimetics of this invention can be further used for the development of even more potent non-peptidic compounds, in addition to their utility as therapeutic compounds.

A number of organizations exist today that are capable of synthesizing the Related Proteins, Related Peptides, and NTP Peptides described herein. For example, given the sequence of an NTP Peptide, the organization can synthesize the peptide and forward the synthesized peptide with accompanying documentation and proof of the identity of the peptide.

This invention also encompasses the use of NTP peptides and their corresponding nucleic acid molecules for assays to test, either qualitatively or quantitatively, for the presence of NTP peptides, NTP peptide DNA or corresponding RNA in mammalian tissue or bodily fluid samples. NTP peptides and their corresponding nucleic acid molecules may have use in the preparation in such assays, whether or not the NTP peptide or the encoded NTP peptide show biological activity. NTP peptide nucleic acid sequence may be a useful source of hybridization probes to test, either qualitatively or quantitatively, for the presence of NTP peptide DNA or corresponding RNA in mammalian tissue or bodily fluid samples.

An NTP peptide which is not in itself biologically active may be useful for preparing antibodies that recognize and/or bind to NTP peptides. Such antibodies may be prepared using standard methods. Thus, antibodies that react with the NTP peptides, as well as short chain antibody fragments and other reactive fragments of such antibodies, are also contemplated as within the scope of the present invention.

The antibodies may be polyclonal, monoclonal, recombinant, chimeric, single-chain and/or bispecific. Typically, the antibody or fragment thereof will either be of human origin, or will be humanized, i.e., prepared so as to prevent or minimize an immune reaction to the antibody when administered to a patient. Preferred
5 antibodies are human antibodies, either polyclonal or monoclonal. The antibody fragment may be any fragment that is reactive with NTP peptides of the present invention, such as, F_{ab}, F_{ab}', etc. Also provided by this invention are the hybridomas generated by presenting any NTP peptide as an antigen to a selected mammal, followed by fusing cells (e.g., spleen cells) of the mammal with certain
10 cancer cells to create immortalized cell lines by known techniques. The methods employed to generate such cell lines and antibodies directed against all or portions of an NTP peptide also are encompassed by this invention.

The antibodies may further be used for *in vivo* and *in vitro* diagnostic or research purposes, such as in labeled form to detect the presence of NTP peptide in
15 a body fluid or cell sample.

This invention also encompasses the use of one or more NTP peptides as calibration standards in assays that test, either qualitatively or quantitatively, for the presence of NTP peptides, NTP peptide DNA or corresponding RNA in mammalian tissue or bodily fluid samples.

20 The present invention is directed to novel methods of treating conditions requiring removal of cells, such as benign and malignant tumors, glandular (e.g. prostate) hyperplasia, unwanted facial hair, warts, and unwanted fatty tissue. Such a method comprises administering to a mammal in need a therapeutically effective amount of NTP peptide.

25 The condition can be, for example, tumors of lung, breast, stomach, pancreas, prostate, bladder, bone, ovary, skin, kidney, sinus, colon, intestine, stomach, rectum, esophagus, blood, brain and its coverings, spinal cord and its coverings, muscle, connective tissue, adrenal, parathyroid, thyroid, uterus, testis, pituitary, reproductive organs, liver, gall bladder, eye, ear, nose, throat, tonsils,
30 mouth, lymph nodes and lymphoid system (e.g., lymphoid tissue), and other organs.

As used herein, the term "malignant tumor" is intended to encompass all forms of human carcinomas, sarcomas and melanomas which occur in the poorly differentiated, moderately differentiated, and well-differentiated forms.

This invention satisfies a need in the art for treatments that can remove
5 benign tumors with less risk and fewer of the undesirable side effects of surgery. A method for removing benign tumors in surgically hazardous areas such as in deep locations in the body (e.g., brain, heart, lungs, and others) is particularly needed.

The method of treating conditions where cells must be removed can be used in conjunction with conventional methods of treating such conditions, such as
10 surgical excision, chemotherapy, and radiation. NTP peptide can be administered before, during, or after such conventional treatments.

The condition to be treated also can be a hyperplasia, hypertrophy, or overgrowth of a tissue selected from the group consisting of lung, breast, stomach, pancreas, prostate, bladder, bone, ovary, skin, kidney, sinus, colon, intestine,
15 stomach, rectum, esophagus, blood, brain and its coverings, spinal cord and its coverings, muscle, connective tissue, adrenal, parathyroid, thyroid, uterus, testis, pituitary, reproductive organs, liver, gall bladder, eye, ear, nose, throat, tonsils, mouth, and lymph nodes and lymphoid system.

Other conditions that can be treated using the method of the invention
20 include, but are not limited to, virally, bacterially, or parasitically altered tissue selected from the group consisting of lung, breast, stomach, pancreas, prostate, bladder, bone, ovary, skin, kidney, sinus, colon, intestine, stomach, rectum, esophagus, blood, brain and its coverings, spinal cord and its coverings, muscle, connective tissue, adrenal, parathyroid, thyroid, uterus, testis, pituitary,
25 reproductive organs, liver, gall bladder, eye, ear, nose, throat, tonsils, mouth, and lymph nodes and lymphoid system.

The condition to be treated can also be a malformation or disorder of a tissue selected from the group consisting of lung, breast, stomach, pancreas, prostate, bladder, bone, ovary, skin, kidney, sinus, colon, intestine, stomach,
30 rectum, esophagus, blood, brain and its coverings, spinal cord and its coverings, muscle, connective tissue, adrenal, parathyroid, thyroid, uterus, testis, pituitary,

reproductive organs, liver, gall bladder, eye, ear, nose, throat, tonsils, mouth, and lymph nodes and lymphoid system.

In particular, the condition to be treated can be tonsillar hypertrophy, prostatic hyperplasia, psoriasis, eczema, dermatoses or hemorrhoids. The condition
5 to be treated can be a vascular disease, such as atherosclerosis or arteriosclerosis, or a vascular disease, such as varicose veins. The condition to be treated also can be a cosmetic modification to a tissue, such as skin, eye, ear, nose, throat, mouth, muscle, connective tissue, hair, or breast tissue.

Therapeutic compositions of NTP peptides are within the scope of the
10 present invention. Such compositions may comprise a therapeutically effective amount of an NTP peptide in admixture with a pharmaceutically acceptable carrier. The carrier material may be water for injection, preferably supplemented with other materials common in solutions for administration to mammals. Typically, an NTP peptide for therapeutic use will be administered in the form of a composition
15 comprising purified NTP peptide in conjunction with one or more physiologically acceptable carriers, excipients, or diluents. Neutral buffered saline or saline mixed with serum albumin are exemplary appropriate carriers. Preferably, the product is formulated as a lyophilizate using appropriate excipients (e.g., sucrose). Other standard carriers, diluents, and excipients may be included as desired.
20 Compositions including buffers known to those having ordinary skill in the art with an appropriate range of pH values, including Tris buffer having a pH of about 7.0-8.5, or acetate buffer having a pH of about 4.0-5.5, which may further include sorbitol or a suitable substitute therefor.

The use of NTP peptides conjugated or linked or bound to an antibody,
25 antibody fragment, antibody-like molecule, or a molecule with a high affinity to a specific tumor marker, such as a cellular receptor, signal peptide or over-expressed enzyme, for targeting to the unwanted cellular elements also is encompassed by the scope of the invention. The antibody, antibody fragment, antibody-like molecule, or molecule with a high affinity to a specific tumor marker is used to target the
30 NTP peptide conjugate to a specific cellular or tissue target. For example, a tumor with a distinctive surface antigen or expressed antigen can be targeted by the

antibody, antibody fragment, or antibody-like binding molecule and the tumor cells can be killed by the NTP peptide. Such an approach using antibody targeting has the anticipated advantages of decreasing dosage, increasing the likelihood of binding to and uptake by the target cells, and increased usefulness for targeting and
5 treating metastatic tumors and microscopic sized tumors.

This invention also encompasses the use of NTP peptides conjugated or linked or bound to a protein or other molecule to form a composition that, upon cleavage at or near the site(s) of the tumor or other unwanted cells by a tumor- or site-specific enzyme or protease or by an antibody conjugate that targets tumor or
10 other unwanted cells, releases the NTP peptide at or near the site(s) of the tumor or other unwanted cells

This invention also encompasses the use of NTP peptides conjugated or linked or bound to a protein or other molecule to form a composition that releases the NTP peptide or some biologically active fragment of the NTP peptide upon
15 exposure of the tissue to be treated to light (as in laser therapies or other photodynamic or photo-activated therapy), other forms of electro-magnetic radiation such as infra-red radiation, ultraviolet radiation, x-ray or gamma ray radiation, localized heat, alpha or beta radiation, ultrasonic emissions, or other sources of localized energy.

20 The NTP peptides may be employed alone, together, or in combination with other pharmaceutical compositions, such as cytokines, growth factors, antibiotics, apoptotic-inducing agents, anti-inflammatories, and/or chemotherapeutic agents as is appropriate for the indication being treated.

This invention also encompasses therapeutic compositions of NTP peptides
25 employing dendrimers, fullerenes, and other synthetic molecules, polymers and macromolecules where the NTP peptide and/or its corresponding DNA molecule is conjugated with, attached to or enclosed in the molecule, polymer or macromolecule, either by itself or in conjunction with other species of molecule such as a tumor-specific marker. For example, U.S. Patent No. 5,714,166,
30 *Bioactive and/or Targeted Dendrimer Conjugates*, provides a method of preparing

and using, *inter alia*, dendritic polymer conjugates composed of at least one dendrimer with a target director(s) and at least one bioactive agent conjugated to it.

This invention also encompasses therapeutic compositions of NTP peptides and/or genes and drug delivery vehicles such as lipid emulsions, micelle polymers, polymer microspheres, electroactive polymers, hydrogels and liposomes.

5 The use of NTP peptides or related genes or gene equivalents transferred to the unwanted cells also is encompassed by the invention. Overexpression of NTP peptide within the tumor can be used to induce the cells in the tumor to die and thus reduce the tumor cell population. The gene or gene equivalent transfer of NTP peptide to treat the unwanted cellular elements is anticipated to have the advantage of requiring less dosage, and of being passed on to the cellular progeny of the targeted cellular elements, thus necessitating less frequent therapy, and less total therapy. This invention also encompasses the transfer of genes that code for a fusion protein containing an NTP peptide to the unwanted cells or neighboring cells where, following the expression of the gene and the production and/or secretion of the fusion protein, the fusion protein is cleaved either by native enzymes or proteases or by a prodrug to release the NTP peptide in, at or near the unwanted cells.

20 The use of cloned recombinant NTP peptide-antibody conjugates; cloned recombinant NTP peptide-antibody fragment conjugates; and cloned recombinant NTP peptide-antibody-like protein conjugates also is encompassed by the invention. The advantages of a cloned NTP peptide combined with targeting conjugate (such as an antibody, antibody fragment, antibody-like molecule, or a molecule with a high affinity to a cancer-specific receptor or other tumor marker) are that such a molecule combines the targeting advantages described above in addition to advantages for manufacturing and standardized production of the cloned conjugated molecule.

25 Solid dosage forms for oral administration include but are not limited to, capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound preferably is admixed with at least one of the following: (a) one or more inert excipients (or carrier), such as sodium citrate or dicalcium phosphate;

(b) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (c) binders, such as carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose and acacia; (d) humectants, such as glycerol; (e) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate; (f) solution retarders, such as paraffin; (g) absorption accelerators, such as quaternary ammonium compounds; (h) wetting agents, such as acetyl alcohol and glycerol monostearate; (i) adsorbents, such as kaolin and bentonite; and (j) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. For capsules, tablets, and pills, the dosage forms may also comprise buffering agents.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active compounds, the liquid dosage forms may comprise inert diluents commonly used in the art, such as water or other solvents, solubilizing agents, and emulsifiers. Exemplary emulsifiers are ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, oils, such as cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, and sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethyleneglycols, fatty acid esters of sorbitan, or mixtures of these substances, and the like.

Besides such inert diluents, the composition also can include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Actual dosage levels of active ingredients in the compositions of the invention may be varied to obtain an amount of NTP peptide that is effective to obtain a desired therapeutic response for a particular composition and method of administration. The selected dosage level therefore depends upon the desired therapeutic effect, the route of administration, the desired duration of treatment, and other factors.

With mammals, including humans, the effective amounts can be administered on the basis of body surface area. The interrelationship of dosages for animals of various sizes, species and humans (based on mg/M² of body surface) is described by E. J. Freireich *et al.*, *Cancer Chemother. Rep.*, 50(4):219 (1966).

- 5 Body surface area may be approximately determined from the height and weight of an individual (*see e.g.*, Scientific Tables, Geigy Pharmaceuticals, Ardsley, N.Y. pp. 537-538 (1970)).

- The total daily dose of the NTP peptide administered to a host may be in single or divided doses. Dosage unit compositions may contain such amounts of
10 such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the body weight, general health, sex, diet, time and route of administration, potency of the administered drug, rates of absorption and excretion, combination with other drugs and the severity of the
15 particular disease being treated.

- A method of administering an NTP peptide composition according to the invention includes, but is not limited to, administering the compounds intramuscularly, orally, intravenously, intraperitoneally, intracerebrally (intraparenchymally), intracerebroventricularly, intratumorally, intralesionally,
20 intradermally, intrathecally, intranasally, intraocularly, intraarterially, topically, transdermally, via an aerosol, infusion, bolus injection, implantation device, sustained release system etc.

- Another method of administering an NTP peptide of the invention is by a transdermal or transcutaneous route. One example of such an embodiment is the
25 use of a patch. In particular, a patch can be prepared with a fine suspension of NTP peptide in, for example, dimethylsulfoxide (DMSO), or a mixture of DMSO with cottonseed oil and brought into contact with the skin of the tumor carrying mammals away from the tumor location site inside a skin pouch. Other mediums or mixtures thereof with other solvents and solid supports would work equally as
30 well. The patch can contain the NTP peptide compound in the form of a solution or a suspension. The patch can then be applied to the skin of the patient, for

example, by means of inserting it into a skin pouch of the patient formed by folding and holding the skin together by means of stitches, clips or other holding devices. This pouch should be employed in such a manner so that continuous contact with the skin is assured without the interference of the mammal. Besides using a skin
5 pouch, any device can be used that ensures the firm placement of the patch in contact with the skin. For instance, an adhesive bandage could be used to hold the patch in place on the skin.

NTP peptide may be administered in a sustained release formulation or preparation. Suitable examples of sustained-release preparations include
10 semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices include polyesters, hydrogels, polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al*, *Biopolymers*, 22: 547-556 [1983]), poly (2-hydroxyethyl-methacrylate) (Langer *et al.*, *J. Biomed. Mater. Res.*, 15: 167-277
15 [1981] and Langer, *Chem. Tech.*, 12: 98-105 [1982]), ethylene vinyl acetate (Langer *et al.*, *supra*) or poly-D(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also may include liposomes, which can be prepared by any of several methods known in the art (e.g., Eppstein *et al.*, *Proc. Natl. Acad. Sci. USA*, 82: 3688-3692 [1985]; EP 36,676; EP 88,046; and EP 143,949).

Another method of administering an NTP peptide of the invention is by
20 direct or indirect infusion of NTP peptide into the tumor or other tissue to be treated. One example of such an embodiment is the direct injection of NTP peptide into the tumor or other tissue to be treated. The treatment may consist of a single injection, multiple injections on one occasion or a series of injections over a period
25 of hours, days or months with the regression or destruction of the tumor or other tissue to be treated being monitored by means of biopsy, imaging or other methods of monitoring tissue growth. The injection into the tumor or other tissue to be treated may be by a device inserted into an orifice such as the nose, mouth, ear, vagina, rectum or urethra or through an incision in order to reach the tumor or
30 tissue *in vivo* and may performed in conjunction with an imaging or optical system such as ultrasound or fibre optic scope in order to identify the appropriate site for

the injection(s). Another example of such an embodiment is the use of a device that can provide a constant infusion of NTP peptide to the tissue over time.

Another method of administering an NTP peptide of the invention is in conjunction with a surgical or similar procedure employed to physically excise, ablate or otherwise kill or destroy tumor or other tissue or cellular elements required or desired to be removed or destroyed wherein an NTP peptide of the invention is administered to the immediate area(s) surrounding the area(s) where the tumor or other tissue was removed in order to destroy or impede the growth of any tumor cells or other cellular elements not removed or destroyed by the procedure

Another method of administering an NTP peptide of the invention is by implantation of a device within the tumor or other tissue to be treated. One example of such an embodiment is the implantation of a wafer containing NTP peptide in the tumor or other tissue to be treated. The wafer releases a therapeutic dose of NTP peptide into the tissue over time. Alternatively or additionally, the composition may be administered locally via implantation into the affected area of a membrane, sponge, or other appropriate material on to which the NTP peptide has been absorbed. Where an implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of the NTP peptide may be directly through the device via bolus, or via continuous administration, or via catheter using continuous infusion.

An alternative method of administration is to introduce one or more copies of an NTP peptide-encoding gene into the cell being targeted and, if necessary, inducing the copy(ies) of the gene to begin producing NTP peptide intracellularly. One manner in which gene therapy can be applied is to use the NTP peptide-encoding gene (either genomic DNA, cDNA, and/or synthetic DNA encoding the NTP peptide (or a fragment, variant, homologue or derivative thereof) which may be operably linked to a constitutive or inducible promoter to form a gene therapy DNA construct. The promoter may be homologous or heterologous to an endogenous NTP peptide-encoding gene, provided that it is active in the cell or tissue type into which the construct will be inserted. Other components of the gene

therapy DNA construct may optionally include, as required, DNA molecules designed for site-specific integration (e.g., endogenous flanking sequences useful for homologous recombination), tissue-specific promoter, enhancer(s) or silencer(s), DNA molecules capable of providing a selective advantage over the
5 parent cell, DNA molecules useful as labels to identify transformed cells, negative selection systems, cell specific binding agents (such as, for example, for cell targeting) cell-specific internalization factors, and transcription factors to enhance expression by a vector as well as factors to enable vector manufacture.

Means of gene delivery to a cell or tissue *in vivo* or *ex vivo* include (but are
10 not limited to) direct injection of bare DNA, ballistic methods, liposome-mediated transfer, receptor-mediated transfer (ligand-DNA complex), electroporation, and calcium phosphate precipitation, as disclosed in, for example, U.S. Pat. Nos. 4,970,154, WO 96/40958, U.S. Pat. No. 5,679,559, U.S. Pat. No. 5,676,954, and U.S. Pat. No. 5,593,875, the disclosures of which are incorporated by reference
15 herein in their entirety. They also include use of a viral vector such as a retrovirus, adenovirus, adeno-associated virus, pox virus, lentivirus, papilloma virus or herpes simplex virus, use of a DNA-protein conjugate and use of a liposome. The use of gene therapy vectors is described, for example, in U.S. Pat. Nos. 5,672,344, U.S. Pat. No. 5,399,346, U.S. Pat. No. 5,631,236, and U.S. Pat. No. 5,635,399, the
20 disclosures of which are incorporated by reference herein in their entirety.

The NTP peptide-encoding gene may be delivered through implanting into patients certain cells that have been genetically engineered *ex vivo*, using methods such as those described herein, to express and secrete the NTP peptide or fragments, variants, homologues, or derivatives thereof. Such cells may be animal
25 or human cells, and may be derived from the patient's own tissue or from another source, either human or non-human. Optionally, the cells may be immortalized or they may be stem cells. However, in order to decrease the chance of an immunological response, it is preferred that the cells be encapsulated to avoid infiltration of surrounding tissues. The encapsulation materials typically are
30 biocompatible, semi-permeable polymeric enclosures or membranes that allow release of the protein product(s) but prevent destruction of the cells by the patient's

immune system or by other detrimental factors from the surrounding tissues. Methods used for membrane encapsulation of cells are familiar to the skilled artisan, and preparation of encapsulated cells and their implantation in patients may be accomplished without undue experimentation. *See, e.g.*, U.S. Pat. Nos. 5 4,892,538; 5,011,472; and 5,106,627, the disclosures of which are incorporated by reference herein in their entirety. A system for encapsulating living cells is described in PCT WO 91/10425. Techniques for formulating a variety of other sustained or controlled delivery means, such as liposome carriers, bio-erodible particles or beads, are also known to those in the art, and are described, for 10 example, in U.S. Pat. No. 5,653,975, the disclosure of which is incorporated by reference herein in their entirety. The cells, with or without encapsulation, may be implanted into suitable body tissues or organs of the patient.

* * * * *

The following examples are provided to illustrate the present invention. It 15 should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. Throughout the specification, any and all references to a publicly available document, including a U.S. patent, are specifically incorporated by reference.

In particular, this invention expressly incorporates by reference the 20 examples contained in pending United States patent application Serial No. 10/092,934, *Methods of Treating Tumors and Related Conditions Using Neural Thread Proteins*, which show that the entire AD7c-NTP protein is an effective agent for causing cell death both *in vitro* in glioma and neuroblastoma cell cultures and *in vivo* in normal rodent muscle tissue, subcutaneous connective tissue, and 25 dermis, and in a variety of different human and non-human origin tumors, including mammary carcinoma, skin carcinoma and papilloma, colon carcinoma, glioma of brain, and others in rodent models. This invention also expressly incorporates by reference the examples contained in United States patent applications Serial No. 10/153334, which show that NTP peptides are effective 30 agents for causing cell death *in vivo* in normal rodent muscle tissue, subcutaneous connective tissue, dermis and other tissue.

Example 1

The purpose of this example was to determine the effect of NTP peptide #7 on tissue at sites of injection.

5 Four normal rats were injected in the skin and subcutaneously, each in four different foci, and in extremity skeletal muscle, each in two different foci, with NTP peptide #7 in saline in quantities of 100 to 400 μ L at concentrations of 0.1-1 mg/mL delivered from plastic syringes through stainless steel 26 gauge needles.

The animals were observed for 24 hours and painlessly sacrificed at 24
10 hours. The individual foci of infiltration were excised, fixed in 10% formalin, embedded in paraffin, and stained and examined by standard histopathological methods.

The controls received saline alone.

Results: Injection of NTP peptide # 7 produced necrosis of tissue at the
15 injection sites. The necrosis was evident in muscle tissue, subcutaneous connective tissue, and dermis at the sites where NTP peptide # 7 was injected. The necrosis correlated with the areas of injection and did not appear to spread far beyond the site of injection.

Apart from the mild areas of inflammation, controls showed no evidence of
20 necrosis or cell loss. The controls showed minimal or absent muscle changes. Control injections had mild to minimal acute inflammation at the injection sites and focal microhemorrhages from the needles.

Example 2

25 The purpose of this example was to determine the effect of NTP peptide #7 on tissue at sites of injection.

Four rats were anesthetized with ether and given NTP peptide #7 by intraprostatic infusion. The injections consisted of 300 μ L of NTP peptide #7 1 mg/mL in PBS pH 7.4. (1.0 mg/kg). Controls received injections of PBS alone or
30 no injection. Rats were painlessly sacrificed after 72 hours. Prostate glands were

dissected, fixed in 10% buffered formalin for 24 hours, embedded in paraffin, sectioned, and stained with H & E. For each animal the entire prostate gland was embedded and sectioned. All stained sections were examined histologically.

Results: Rat prostate treated with NTP peptide #7 showed necrosis of tissue
5 at the injection sites with loss of glandular epithelium, flattening and atrophy. There was no discernible difference between control PBS injections alone, and controls with no injections.

Example 3

10 The purpose of this example was to determine the effect of NTP peptide #14 on tissue at sites of injection.

Rats were injected in the skin and subcutaneously as in Example 1 above, except they were injected with NTP peptide #14.

15 The animals were observed for 24 hours and painlessly sacrificed at 24 hours. Tissues were excised, fixed in 10% formalin, embedded in paraffin, and stained and examined by standard histopathological methods.

The controls were the same as Example 1.

Results: Injection of NTP peptide #14 produced cell death and necrosis of tissue at the injection sites. Similar to Example 1 above, the cell death was present
20 in muscle tissue, subcutaneous connective tissue, and dermis at the sites where NTP peptide #14 was injected.

Apart from the mild areas of inflammation, controls showed minimal evidence of necrosis or cell loss. Control injections had mild to minimal acute inflammation at the injection sites and occasional focal microhemorrhages from the
25 needles.

Example 4

The purpose of this example was to determine the effect of NTP peptide #17 on tissue at sites of injection.

30 Normal rats were injected in the prostate as in the above Example 2, except they were injected with NTP peptide #17. Rats were painlessly sacrificed after 72 hours and their prostate glands were examined as in Example 2.

Results: As in the above Example 2, injection of NTP peptide #17 produced significant cell loss and atrophy in the prostate at 72 hours. Controls showed minimal or absent changes, consisting of mild focal inflammation from the
5 needles.

* * *

The invention has been described with reference to particularly preferred embodiments and examples. Those skilled in the art will appreciate, however, that
10 various modifications may be made to the invention without departing from the spirit and scope thereof.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A peptide comprising at least one peptide selected from group consisting of:
 - a) The peptide represented by the amino acid sequence in SEQ ID NO. 10 (Pro-Gly-Phe-Phe-Lys-Leu-Phe-Ser-Cys-Pro-Ser-Leu-Leu-Ser-Ser-Trp-Asp-Tyr-Arg-Arg);
 - b) The peptide represented by the amino acid sequence in SEQ ID NO. 22 (Ser-Ser-Trp-Asp-Tyr-Arg-Arg-Phe-Ile-Leu-Phe-Phe-Leu);
 - c) The peptide represented by the amino acid sequence in SEQ ID NO. 23 (Trp-Asp-Tyr-Arg-Arg-Phe-Ile-Phe-Asn-Phe-Leu);
 - d) The peptide represented by the amino acid sequence in SEQ ID NO. 24 (Phe-Asn-Phe-Cys-Leu-Phe);
 - e) The peptide represented by the amino acid sequence in SEQ ID NO. 25 (Phe-Ile-Phe-Asn-Phe-Leu); and
 - f) The peptide represented by the amino acid sequence in SEQ ID NO. 26 (Pro-Ala-Ser-Ala-Ser-Pro-Val-Ala-Gly-Ile-Thr-Gly-Met);
 wherein said peptide is effective as an agent for the destruction or removal of unwanted or harmful cells.
2. A peptide consisting of at least one peptide selected from:
 - a) the peptide represented by the amino acid sequence in SEQ ID NO. 19 (Leu-Pro-Ser-Ser-Trp-Asp-Tyr-Arg-Arg); and
 - b) the peptide represented by the amino acid sequence in SEQ ID NO. 28 (Pro-Ala-Ser-Ala-Ser-Gln-Ser-Ala-Gly-Ile-Thr-Gly-Val)
 wherein said peptide is effective as an agent for the destruction or removal of unwanted or harmful cells.
3. A composition comprising one or more peptides according to claim 1 or claim 2 and a carrier therefor.
4. A peptide comprising a homologue, derivative, fragment or variant of the peptide according to claim 1 or claim 2, wherein the homologue, derivative, fragment or variant is effective as an agent for the destruction or removal of unwanted or harmful cells.

5. A peptide comprising an amino acid in a reverse-D order based on the amino acid sequence for the peptide according to any one of claims 1, 2 or 4.
6. A peptide comprising a peptide according to any one of claims 1, 4 or 5
5 modified by from one to 25 additional amino acids flanking either the 3' or 5' end of the peptide.
7. A peptide comprising at least two peptides according to any one of claims 1, 2
10 or 4 to 6.
8. A peptide comprising at least two repetitions of the peptide according to any one of claims 1, 2 or 4 to 6.
9. A mimetic of a peptide according to any one of claims 1, 2 or 4 to 8.
15
10. A peptide comprising a peptide according to any one of claims 1, 2 or 4 to 8 fused to an antibody, fragment of an antibody or an antibody-like molecule.
11. A nucleic acid encoding an amino acid sequence corresponding to a peptide
20 according to claim 1 or claim 2 and homologues, fragments and variants thereof wherein the homologues, fragments and variants are effective as agents for the destruction or removal of unwanted or harmful cells.
12. A composition comprising one or more nucleic acids according to claim 11 and
25 a pharmaceutically acceptable carrier therefor.
13. A method of treating a condition in a patient requiring removal or destruction of cells comprising administering to a mammal in need a therapeutically effective amount of a peptide according to any one of claims 1, 2, 4 to 8 or 10.
30
14. The method according to claim 13, wherein administering is comprised of a method selected from the group consisting of orally, subcutaneously, intradermally, intranasally, intravenously, intramuscularly, intrathecally, intranasally, intratumorally, topically, and transdermally.
35

15. The method of claim 13 or claim 14, wherein the treatment is administered to the patient before, during, or after the patient has or will be treated with a treatment selected from the group of treatments consisting of surgical excision, transplantation, grafting, chemotherapy, immunotherapy, vaccination, thermal or electrical ablation, cryotherapy, laser therapy, phototherapy, gene therapy, and radiation.
5
16. The method of any one of claims 13 to 15, where the condition is selected from the group consisting of a benign tumor of a tissue, a malignant tumor of a tissue, a hyperplasia of a tissue, a hypertrophy of a tissue, an overgrowth of a tissue, a virally altered tissue, a bacterially altered tissue, a parasitically altered tissue, or a malformation of a tissue; wherein the tissue is selected from the group consisting of lung, breast, stomach, pancreas, prostate, bladder, bone, ovary, skin, kidney, sinus, colon, intestine, stomach, rectum, esophagus, heart, spleen, salivary gland, blood, brain and its coverings, spinal cord and its coverings, muscle, connective tissue, adrenal, parathyroid, thyroid, uterus, testis, pituitary, reproductive organs, liver, gall bladder, eye, ear, nose, throat, tonsils, mouth, lymph nodes, and lymphoid tissue.
10
15
17. The method of claim 16, wherein the tissue is lymphoid tissue, skin, eye, ear, nose, throat, mouth, muscle, connective, hair, or breast.
20
18. The method of claim 16, wherein the condition is tonsillary hypertrophy.
19. The method of claim 18, wherein the condition is the vascular disease atherosclerosis or arteriosclerosis.
25
20. The method of any one of claims 13 to 15, wherein the condition is one of an inflammatory disease, autoimmune disease, metabolic disease, hereditary/genetic disease, traumatic disease or physical injury, nutritional deficiency disease, infectious disease, amyloid disease, fibrosis disease, storage disease, congenital malformation, enzyme deficiency disease, poisoning, intoxication, environmental disease, radiation disease, endocrine disease, degenerative disease and mechanical disease.
30
21. The method of any one of claims 13 to 20, wherein said peptide is derived from the amino acid sequence for a pancreatic thread protein.
35

- 5 22. The method of any one of claims 13 to 21, wherein the peptide is conjugated, linked, or bound to a molecule selected from the group consisting of an antibody, antibody fragment, and an antibody-like binding molecule, wherein the molecule has a higher affinity for binding to a tumor or other target than binding to other cells.
- 10 23. The method of any one of claims 13 to 21, wherein the peptide is part of a single new cloned recombinant molecule consisting of the peptide and a molecule selected from the group consisting of an antibody, antibody fragment, and antibody-like binding molecule, wherein the molecule has a higher affinity for binding to a tumor or other target than binding to other cells.
- 15 24. Use of a peptide according to any one of claims 1, 2, 4 to 8 or 10 for the manufacture of a medicament for treating a condition in a patient requiring removal or destruction of cells.

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Figure 1

AD7C-NTP [SEQ ID NO. 1]

1	tttttttttttgag	ATG GAG TTT TCG CTC TTG TTG CCC AGG CTG GAG TGC AAT GGC GCA ATC	62
1		M E F S L L L P R L R C N G A I	16
63	TCA GCT CAC CGC AAC CTC CGC CTC CCG GGT TCA AGC GAT TCT CCT GCC TCA GGC TCC CCA	122	
17	S A H R N L R L P G S S D S P A S A S P	36	
123	GTA GCT GGG ATT ACA GGC ATG TGC ACC CAC GCT CGG CTA ATT TTG TAT TTT TTT TTA GTA	182	
37	V A G I T G M C T H A R L I L Y F F L V	56	
183	GAG ATG GAG TTT CTC CAT GTT GGT CAG GCT GGT CTC GAA CTC CCG ACC TCA GAT GAT CCC	242	
57	E M E F L H V G Q A G L E L P T S D D P	76	
243	TCC GTC TCG GCC TCC CAA AGT GCT AGA TAC AGC ACT GGC CAC CAT CCC CGG CTC TGC CTG	302	
77	S V S A S Q S A R Y R T G H H A R L C L	96	
303	GCT AAT TTT TGT GGT AGA AAC AGG GTT TCA CTG ATG TGC CCA AGC TGG TCT CCT GAG CTC	362	
97	A N F C G R N R V S L M C P S W S P E L	116	
363	AAG CAG TCC ACC TGC CTC AGC CTC CCA AAG TCC TGC GAT TAC AGG CGT GCA GCC GTG CCT	422	
117	K Q S T C L S L P K C W D Y R R A A V P	136	
423	GGC CTT TTT ATT TTA TTT TTT TTA AGA CAC AGG TGT CCC ACT CTT ACC CAG GAT GAA GTG	482	
137	G L F I L F F L R H R C P T L T Q D E V	156	
483	CAG TGG TGT GAT CAC AGC TCA CTG CAG CCT TCA ACT CCT GAC ATC AAG CAT CCT CCT GCC	542	
157	Q W C D H S S L Q P S T P E C K H P P A	176	
543	TCA GCC TCC CAA GTA GCT GGG ACC AAA GAC ATG CAC CAC TAC ACC TGG CTA ATT TTT ATT	602	
177	S A S Q V A G T K D M H H Y T W L I F I	196	
603	TTT ATT TTT AAT TTT TTG AGA CAG AGT CTC AAC TCT GTC ACC CAG GCT GCA GTG CAG TGG	662	
197	F I F N F L R Q S L N S V T Q A G V Q W	216	
663	CGC AAT CTT GGC TCA CTG CAA CCT CTG CCT CCC GGG TTC AAG TTA TTC TCC TGC CCC AGC	722	
217	R N L G S L Q P L P P G F K L F S C P S	236	
723	CTC CTG AGT AGC TGG GAC TAC AGG CGC CCA CCA CGC CTA GCT AAT TTT TTT GTA TTT TTA	782	
237	L L S S W D Y R R P P R L A N F F V F L	256	
783	GTA GAG ATG GGG TTC ACC ATG TTC GCC AGG TTG ATC TTG ATC TCT GGA CCT TGT GAT CTG	842	
257	V E M G F T M F A R L C L I S G P C D L	276	
843	CCT GCC TCG GCC TCC CAA AGT GCT GGG ATT ACA GGC GTG AGC CAC CAC GCC CGG CTT ATT	902	
277	P A S A S Q S A G I T G V S H H A R L I	296	
903	TTT AAT TTT TGT TTG TTT GAA ATG GAA TCT CAC TCT GTT ACC CAG GCT GGA GTG CAA TGG	962	
297	F N F C L F E M E S H S V T Q A G V Q W	316	
963	CCA AAT CTC GGC TCA CTG CAA CCT CTG CCT CCC GGG CTC AAG CGA TTC TCC TGT CTC AGC	1022	
317	P N L G S L Q P L P P G L K R P S C L S	336	
1023	CTC CCA AGC ACC TGG GAT TAC GGG CAC CTG CCA CCA CAC CCC GCT AAT TTT TGT ATT TTC	1082	
337	L P S S W D Y G H L P P H P A N F C I F	356	
1083	ATT AGA GGC GGG GTT TCA CCA TAT TTG TCA GGC TGG TCT CAA ACT CCT GAC CTC AGG tgac	1143	
357	I R C G V S P Y L S G W S Q T P D L R	375	
1144	ccacctgcctcagccttccaaagtctgtgggattacaggcgtgagccacctcaccagcggcctaatttagataaaaaaat	1223	
1224	atgtagcaatgggggtcttctgtatgttgcacaggctgggtctcaaaactctgtgcttcatgcaatccttccaaatgagcca	1303	
1304	caacacccagccagtcacattttttaaacagttacatctttatttttagtataactagaagtaatacaataaacatgtcca	1383	
1384	aactgcacattcagtagtaaacagagttcttttataacttttaacaaagcttttagagca	1442	

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Figure 2

NTP[122] [SEQ ID NO. 2]

```
1   Met-Met-Val-Cys-Trp-Asn-Arg-Phe-Gly-Lys-
    M  M  V  C  W  N  R  F  G  K

11  Trp-Val-Tyr-Phe-Ile-Ser-Ala-Ile-Phe-Asn-
    W  V  Y  F  I  S  A  I  F  N

21  Phe-Gly-Pro-Arg-Tyr-Leu-Tyr-His-Gly-Val-
    F  G  P  R  Y  L  Y  H  G  V

31  Pro-Phe-Tyr-Phe-Leu-Ile-Leu-Val-Arg-Ile-
    P  F  Y  F  L  I  L  V  R  I

41  Ile-Ser-Phe-Leu-Ile-Gly-Asp-Met-Glu-Asp-
    I  S  F  L  I  G  D  M  E  D

51  Val-Leu-Leu-Asn-Cys-Thr-Leu-Leu-Lys-Arg-
    V  L  L  N  C  T  L  L  K  R

61  Ser-Ser-Arg-Phe-Arg-Phe-Trp-Gly-Ala-Leu-
    S  S  R  F  R  F  W  G  A  L

71  Val-Cys-Ser-Met-Asp-Ser-Cys-Arg-Phe-Ser
    V  C  S  M  D  S  C  R  F  S

81  Arg-Val-Ala-Val-Thr-Tyr-Arg-Phe-Ile-Thr-
    R  V  A  V  T  Y  R  F  I  T

91  Leu-Leu-Asn-Ile-Pro-Ser-Pro-Ala-Val-Trp-
    L  L  N  I  P  S  P  A  V  W

101 Met-Ala-Arg-Asn-Thr-Ile-Asp-Gln-Gln-Val-
    M  A  R  N  T  I  D  Q  Q  V

111 Leu-Ser-Arg-Ile-Lys-Leu-Glu-Ile-Lys-Arg-
    L  S  R  I  K  L  E  I  K  R

121 Cys-Leu
    C  L
```


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Figure 3

NTP[112] [SEQ ID NO. 3]

```
1   Met-Ala-Gln-Ser-Arg-Leu-Thr-Ala-The-Ser-
    M  A  Q  S  R  L  T  A  T  S
11  Ala-Ser-Arg-Val-Gln-Ala-Ile-Leu-Leu-Ser-
    A  S  R  V  Q  A  I  L  L  S
21  Gln-Pro-Pro-Lys-Gln-Leu-Gly-Leu-Arg-Ala-
    Q  P  P  K  Q  L  G  L  R  A
31  Pro-Ala-Asn-Thr-Pro-Leu-Ile-Phe-Val-Phe-
    P  A  N  T  P  L  I  F  V  F
41  Ser-Leu-Glu-Ala-Gly-Phe-His-His-Ile-Cys-
    S  L  E  A  G  F  H  H  I  C
51  Gln-Ala-Gly-Leu-Lys-Leu-Leu-Thr-Ser-Gly-
    Q  A  G  L  K  L  L  T  S  G
61  Asp-Pro-Pro-Ala-Ser-Ala-Phe-Gln-Ser-Ala-
    D  P  P  A  S  A  F  Q  S  A
71  Gly-Ile-Thr-Gly-Val-Ser-His-Leu-Thr-Gln-
    G  I  T  G  V  S  H  L  T  Q
81  Pro-Ala-Asn-Leu-Asp-Lys-Lys-Ile-Cys-Ser-
    P  A  N  L  D  K  K  I  C  S
91  Asn-Gly-Gly-Ser-Cys-Tyr-Val-Ala-Gln-Ala-
    N  G  G  S  C  Y  V  A  Q  A
101 Gly-Leu-Lys-Leu-Leu-Ala-Ser-Cys-Asn-Pro-
    G  L  K  L  L  A  S  C  N  P
111 Ser-Lys
    S  K
```

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Figure 4

NTP[106] [SEQ ID NO. 4]

```
1   Met-Trp-Thr-Leu-Lys-Ser-Ser-Leu-Val-Leu-
    M   W   T   L   K   S   S   L   V   L
11  Leu-Leu-Cys-Leu-Thr-Cys-Ser-Tyr-Ala-Phe-
    L   L   C   L   T   C   S   Y   A   F
21  Met-Phe-Ser-Ser-Leu-Arg-Gln-Lys-Thr-Ser-
    M   F   S   S   L   R   Q   K   T   S
31  Glu-Pro-Gln-Gly-Lys-Val-Pro-Cys-Gly-Glu-
    E   P   Q   G   K   V   P   C   G   E
41  His-Phe-Arg-Ile-Arg-Gln-Asn-Leu-Pro-Glu-
    H   F   R   I   R   Q   N   L   P   E
51  His-Thr-Gln-Gly-Trp-Leu-Gly-Ser-Lys-Trp-
    H   T   Q   G   W   L   G   S   K   W
61  Leu-Trp-Leu-Leu-Phe-Ala-Val-Val-Pro-Phe-
    L   W   L   L   F   A   V   V   P   F
71  Val-Ile-Leu-Lys-Cys-Gln-Arg-Asp-Ser-Glu-
    V   I   L   K   C   Q   R   D   S   E
81  Lys-Asn-Lys-Val-Arg-Met-Ala-Pro-Phe-Phe-
    K   N   K   V   R   M   A   P   F   F
91  Leu-His-His-Ile-Asp-Ser-Ile-Ser-Gly-Val-
    L   H   H   I   D   S   I   S   G   V
101 Ser-Gly-Lys-Arg-Met-Phe
    S   G   K   R   M   F
```

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Figure 5

NTP [106] [SEQ ID NO. 5]

```
1  Met-Phe-Phe-Val-Leu-Tyr-Arg-Phe-Cys-Phe-
   M F F V L Y R F C F
11 Cys-Phe-Phe-Glu-Thr-Glu-Ser-His-Ser-Leu-
   C F F E T E S H S L
21 Thr-Gln-Ala-Gly-Val-Gln-Trp-Cys-Glu-Leu-
   T Q A G V Q W C E L
31 Gly-Ser-Pro-Gln-Pro-Leu-Pro-Ser-Gly-Phe-
   G S P Q P L P S G F
41 Lys-Arg-Phe-Ser-Cys-Leu-Ser-Leu-Leu-Ser-
   K R F S C L S L L S
51 Ser-Trp-Asp-Tyr-Ser-His-Glu-Pro-Pro-His-
   S W D Y S H E P P H
61 Pro-Val-Ile-Cys-Ser-Phe-Leu-Met-Glu-Lys-
   P V I C S F L M E K
71 Cys-Leu-Ile-Leu-Tyr-Lys-Pro-Asn-Gly-Asp-
   C L I L Y K P N G D
81 Thr-Ile-Gly-Pro-Ile-Leu-Val-Gln-Gln-Gly-
   T I G P I L V Q Q G
91 Lys-Arg-Gln-Lys-Leu-Tyr-Ile-Ser-Ala-Asp-
   K R Q K L Y I S A D
100 Leu-Val-His-Leu-Ile-Ala
    L V H L I A
```

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Figure 6

NTP[98] [SEQ ID NO. 6]

```
1   Glu-Ala-Tyr-Tyr-Thr-Met-Leu-His-Leu-Pro-
    E  A  Y  Y  T  M  L  H  L  P

11  Thr-Thr-Asn-Arg-Pro-Lys-Ile-Ala-His-Cys
    T  T  N  R  P  K  I  A  H  C

21  Ile-Leu-Phe-Asn-Gln-Pro-His-Ser-Pro-Arg-
    I  L  F  N  Q  P  H  S  P  R

31  Ser-Asn-Ser-His-Ser-His-Pro-Asn-Pro-Leu-
    S  N  S  H  S  H  P  N  P  L

41  Lys-Leu-His-Arg-Arg-Ser-His-Ser-His-Asn-
    K  L  H  R  R  S  H  S  H  N

51  Arg-Pro-Arg-Ala-Tyr-Ile-Leu-Ile-Thr-Ile-
    R  P  R  A  Y  I  L  I  T  I

61  Leu-Pro-Ser-Lys-Leu-Lys-Leu-Arg-Thr-His-
    L  P  S  K  L  K  L  R  T  H

71  Ser-Gln-Ser-His-His-Asn-Pro-Leu-Ser-Arg-
    S  Q  S  H  H  N  P  L  S  R

81  Thr-Ser-Asn-Ser-Thr-Pro-Thr-Asn-Ser-Phe-
    T  S  N  S  T  P  T  N  S  F

91  Leu-Met-Thr-Ser-Ser-Lys-Pro-Arg
    L  M  T  S  S  K  P  R
```

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Figure 7

NTP[75] [SEQ ID NO. 7]

```
1   Ser-Ser-Ser-Leu-Gly-Leu-Pro-Lys-Cys-Trp-  
    S  S  S  L  G  L  P  K  C  W  
11  Asp-Tyr-Arg-His-Glu-Leu-Leu-Ser-Leu-Ala-  
    D  Y  R  H  E  L  L  S  L  A  
21  Leu-Met-Ile-Asn-Phe-Arg-Val-Met-Ala-Cys  
    L  M  I  N  F  R  V  M  A  C  
31  Thr-Phe-Lys-Gln-His-Ile-Glu-Leu-Arg-Gln-  
    T  F  K  Q  H  I  E  L  R  Q  
41  Lys-Ile-Ser-Ile-Val-Pro-Arg-Lys-Leu-Cys-  
    K  I  S  I  V  P  R  K  L  C  
51  Cys-Met-Gly-Pro-Val-Cys-Pro-Val-Lys-Ile-  
    C  M  G  P  V  C  P  V  K  I  
61  Ala-Leu-Leu-Thr-Ile-Asn-Gly-His-Cys-Thr-  
    A  L  L  T  I  N  G  H  C  T  
71  Trp-Leu-Pro-Ala-Ser  
    W  L  P  A  S
```

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Figure 8

NTP[68] [SEQ ID NO. 8]

```
1  Met-Phe-Val-Phe-Cys-Leu-Ile-Leu-Asn-Arg-  
   M  F  V  F  C  L  I  L  N  R  
  
11 Glu-Lys-Ile-Lys-Gly-Gly-Asn-Ser-Ser-Phe-  
   E  K  I  K  G  G  N  S  S  F  
  
21 Phe-Leu-Leu-Ser-Phe-Phe-Phe-Ser-Phe-Gln-  
   F  L  L  S  F  F  F  S  F  Q  
  
31 Asn-Cys-Cys-Gln-Cys-Phe-Gln-Cys-Arg-Thr-  
   N  C  C  Q  C  F  Q  C  R  T  
  
41 Thr-Glu-Gly-Tyr-Ala-Val-Glu-Cys-Phe-Tyr-  
   T  E  G  Y  A  V  E  C  F  Y  
  
51 Cys-Leu-Val-Asp-Lys-Ala-Ala-Phe-Glu-Cys-  
   C  L  V  D  K  A  A  F  E  C  
  
61 Trp-Trp-Phe-Tyr-Ser-Phe-Asp-Thr  
   W  W  F  Y  S  F  D  T
```

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Figure 9

NTP[61] [SEQ ID NO. 9]

```
1  Met-Glu-Pro-His-Thr-Val-Ala-Gln-Ala-Gly-
   M E P H T V A Q A G
11 Val-Pro-Gln-His-Asp-Leu-Gly-Ser-Leu-Gln-
   V P Q H D L G S L Q
21 Ser-Leu-Leu-Pro-Arg-Phe-Lys-Arg-Phe-Ser-
   S L L P R F K R F S
31 Cys-Leu-Ile-Leu-Pro-Lys-Ile-Trp-Asp-Tyr-
   C L I L P K I W D Y
41 Arg-Asn-Met-Asn-Thr-Ala-Leu-Ile-Lys-Arg-
   R N M N T A L I K R
51 Asn-Arg-Tyr-Thr-Pro-Glu-Thr-Gly-Arg-Lys-
   N R Y T P E T G R K
61 Ser
   S
```